Biomarkers to improve prognostication in heart failure

Laura C. van Vark

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Biomarkers to Improve Prognostication in Heart Failure

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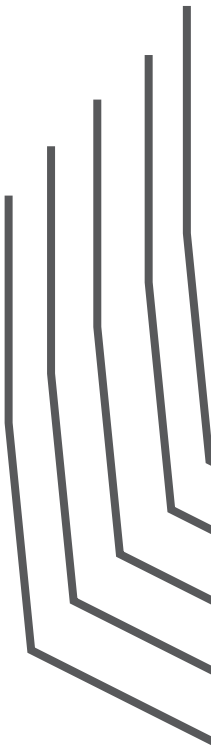

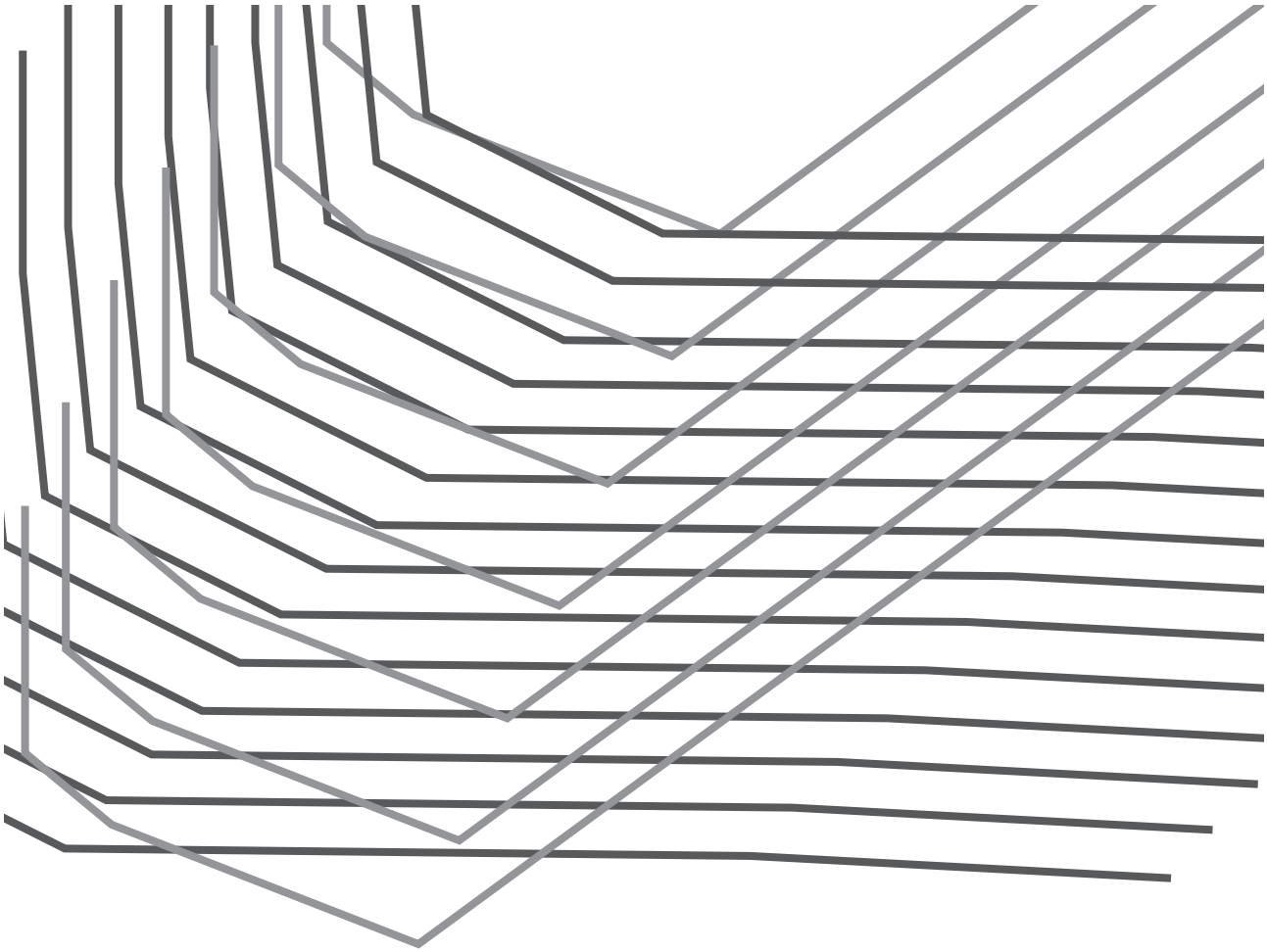

Chapter 1

General introduction and outline of thesis

Biomarkers and heart failure

Heart failure (HF) is a major and growing public health problem.[1] Prevalence of HF is high, especially in the elderly, as about 10% of men and 8% of women over the age of 60 years are affected, in industrialized countries.[1,2] Moreover, in the Rotterdam study, a population-based cohort study, the lifetime risk of developing HF was 33% for men and 29% for women at the age of 55.[3] Despite the significant advances in therapies and prevention, mortality and morbidity is still high and quality of life poor.

HF is a clinical syndrome characterized by typical symptoms that may be accompanied by signs caused by a structural or functional cardiac abnormality, resulting in a reduced cardiac output or elevated intracardiac pressures at rest or during stress.[4] HF is complex involving several underlying pathophysiological processes.[2] Furthermore, HF is a progressive disease. HF patients are in need of permanent medication use and acute decompensation is common, in which case advanced treatment and hospitalization is necessary. It is essential to improve prognostication in heart failure patients to be able to help physicians diagnose patients early and anticipate in time to intensify heart failure treatment and patient monitoring.[5]

A biomarker is a measurable indicator reflecting a biological state, for example a substance measured in the blood. In heart failure biomarkers may reflect mechanisms such as inflammation, oxidative stress, neurohormonal activation, myocardial and matrix remodelling.[5] There is growing evidence that biomarkers released as a consequence of these underlying pathophysiological processes may not only improve our pathophysiological knowledge, but are also useful for identification of healthy people who are at risk for developing HF, for the diagnosis of HF patients and for improved risk prediction in HF patients.[2] Circulating microRNAs have been proposed as an attractive new class of biomarkers, because of their association with different heart failure aetiologies and disease progression, their stability in the circulation, and their ensuing reliable assessment in easily accessible samples.[6] Due to different heart failure aetiologies, different underlying pathophysiological mechanisms and disease progression over time, one single biomarker measurement may not adequately reflect the HF disease. First of all, combining different biomarkers is proposed to be useful for providing additional risk stratification.[5] Secondly, multiple biomarker measurements over time are assumed to propose additional information.[7] Thirdly, certain biomarkers may be useful in specific heart failure populations, such as HF with a preserved ejection fraction (HFpEF).[8]

Health related quality of life in heart failure patients

From a patient's perspective, quality of life is very important. Studies have found that patients value quality of life at least as important as longevity.[9,10] Among HF patients, health-related quality of life (HRQoL) is worse compared to the general population, and is worse than that of patients with other chronic conditions.[10-12] Moreover, an impaired HRQoL is also related to adverse outcome.[13]

It is known that HRQoL is influenced by the occurrence of HF symptoms.[14, 15] HF symptoms are more prevalent in depressed patients, which is a common comorbidity in HF patients.[16] Therefore, more insight is needed in the relation between depression and heart failure symptoms. Besides symptoms of depression, the presence of non-cardiac comorbidities may also impact HRQoL. Determinants related to HRQoL may be influenced to improve heart failure treatment.

Treatment effect of raas inhibitors in hypertensive patients

The renin-angiotensin-aldosteron system (RAAS) plays an important role in the regulation of hemodynamic stability in the human body by controlling electrolytes and fluid balance. The RAAS functions through direct and indirect effects on several organ systems and interacts with autonomic nervous system and several vasoactive hormones.[17, 18] Excessive stimulation of the RAAS causes pathologic changes in a wide variety of organ systems. Overactive RAAS is associated with hypertension, renal injury, atherosclerosis and left ventricular dysfunction.[19, 20] Therefore the blockade of RAAS has become a key therapeutic target in a wide variety of patients, such as hypertensive patients and RAAS blockade is also a cornerstone in treatment of heart failure patients. The clinically most important examples of pharmacologic agents that block the RAAS currently are the angiotensin-converting enzyme (ACE) inhibitors and AT1 receptor blockers (ARBs).

Reductions in both cardiovascular morbidity and mortality have been well demonstrated with RAAS inhibitors across specific populations, such as heart failure patients, high cardiovascular risk patients and stable coronary disease patients.[21-23] In these trials, patients were selected for a criterion other than hypertension per se, and so only half of the patients enrolled in these trials had prevalent hypertension. The beneficial effects of RAAS inhibitors in hypertensive patients on mortality have not been convincingly demonstrated, as most trials were underpowered for this endpoint.

Aim and outline of this thesis

Our main objective in this thesis is to evaluate biomarkers that reflect the underlying pathophysiological processes in HF, such as mechanical overload, atherosclerosis, inflammation and cardiac fibrosis.[2] To take into account the dynamic and progressive nature of HF over time, we designed the TRanslational Initiative on Unique and novel strategies for Management of Patients with Heart failure (TRIUMPH) study.[24] TRIUMPH is a multi-centre observational cohort study in acute HF patients. Patients were enrolled when they where hospitalized with decompensation of known chronic HF or newly diagnosed HF. In TRIUMPH, biomarkers were evaluated for their prognostic properties using a unique design of seven planned repeated measurements during 1-year follow-up. To be able to account for repeated biomarker measurements in our analyses, joint models were used.

Based on previous clinical and epidemiological studies, ST2 and galectin-3 were marked as biomarkers with high potential for improving prognostication.[7,25] Although the AHA/ACC Guidelines advise physicians to consider using ST2 and galectin-3 as an additional biomarker to natriuretic peptide levels for prognostication in HF patients,[26] the ESC guidelines state that none of the newer biomarkers has reached the stage of being recommended for routine use.[4] Therefore in **chapter two, three** and **four**, ST2 and galectin-3 were extensively evaluated. Subsequently, we performed a multi-marker, multi-time point analyses, to assess the independent prognostic value of repeated measurements of NT-proBNP, cardiac troponin I, ST2, galectin-3 and creatinine for all-cause and cardiovascular mortality (**chapter five**).

Hence, we evaluated the potential for prognostication of serial measured microRNAs. First, an RNA sequencing discovery experiment in pigs was used to identify the most promising novel microRNA (miR-1306-5p). Secondly, multiple miRs known to be cardiac-enriched or previously linked to HF were evaluated (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) (**chapter six**).

Elevation of inflammatory markers have also been associated with an increase of the development of HF.[27] Lipoprotein phospholipase A2 (Lp-PLA2) is proposed as a pro-inflammatory marker and is an independent predictor of cardiovascular disease.[28] We assess the association between Lp-PLA2, a pro-inflammatory biomarker, and incident HF in a random sample of the Rotterdam Study, a population based cohort study among persons aged 55 years and over (**chapter seven**).[29] In **chapter eight**, we reviewed the

associations of several biomarkers with the occurrence and prognosis of HF with a preserved ejection fraction.

The aim of the second part of this thesis is to evaluate symptom burden and health-related quality of life (HRQoL) determinants in acute HF patients. We evaluated the occurrence and burden of HF symptoms in acute HF patients with and without depression, based on data from the TRIUMPH cohort (**chapter nine**). Secondly, we investigated the relation between presence of non-cardiac comorbidities (diabetes mellitus, chronic kidney dysfunction, COPD or prior CVA) and HRQoL. Furthermore, we evaluated determinants of HRQoL in acute HF patients, with and without comorbidities (**chapter ten**).

In the final part of this thesis we examined whether RAAS inhibitors reduce all-cause and cardiovascular mortality in hypertensive patients. We considered RAAS inhibitors as a class of drugs, as well as ACE inhibitors and ARBs separately (**chapter eleven**). In addition, we assessed the effectiveness of RAAS inhibitors to prevent all-cause mortality and cardiovascular death, myocardial infarction and stroke in hypertensive patients. We performed a 'number needed to treat' analyses to give insight to the absolute treatment effect of RAAS inhibitors instead of relative risk reduction alone, which is considered an important measure to accurately communicate risk (**chapter twelve**).[30,31]

Finally, in chapter thirteen, a general overview and discussion of all results described in this thesis is given.

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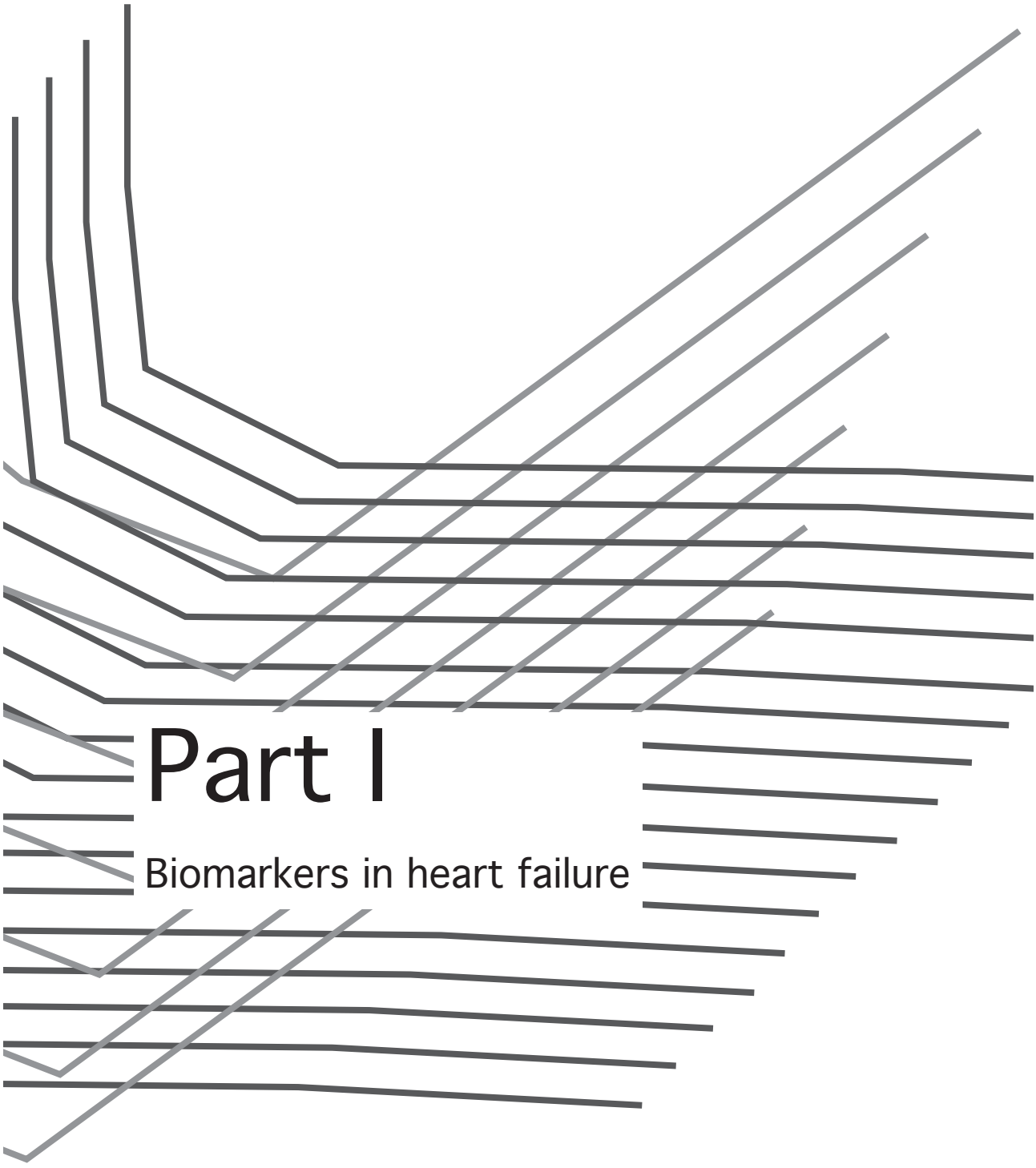

Part I

Biomarkers in heart failure

Chapter 2

Prognostic value of serial galectin-3 measurements in patients with acute heart failure

L.C. van Vark, I. Lesman-Leegte, S.J. Baart, D. Postmus, Y.M. Pinto, R.A. de Boer, F.W. Asselbergs, E.M.C.J. Wajon, J.G. Orsel, E. Boersma, H.L. Hillege, K.M. Akkerhuis; TRIUMPH Investigators

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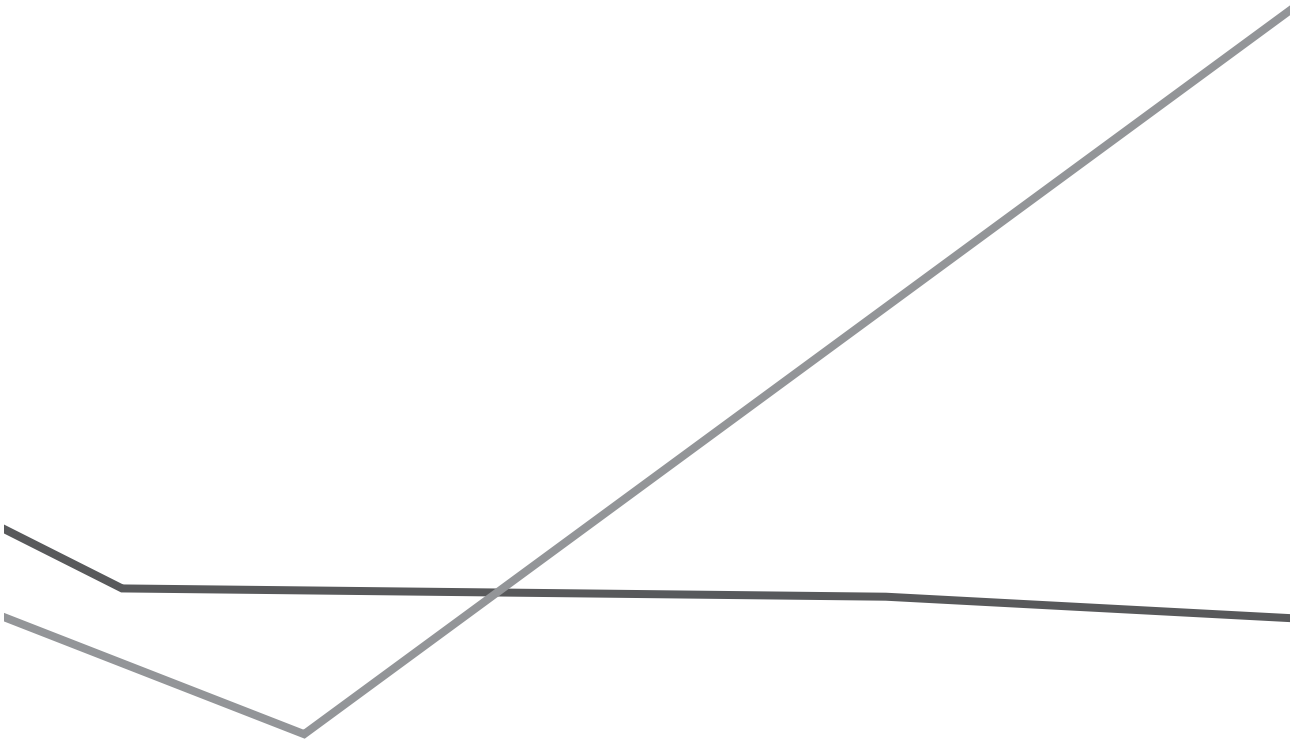

Abstract

Background

Several clinical studies have evaluated the association between galectin-3 levels and outcome in patients with heart failure (HF). However, only little is known about the predictive value of repeated galectin-3 measurements. This study evaluates the prognostic value of repeated time-dependent galectin-3 measurements in acute HF patients.

Methods

In the TRIUMPH clinical cohort study, 496 acute HF patients were enrolled in 14 hospitals in The Netherlands, between 2009 and 2014. Repeated blood samples (seven) were drawn during 1-year follow-up. Associations between repeated biomarker measurements and the primary endpoint were assessed using a joint model.

Results

Median age was 74 years and 37% were women. The primary endpoint, composite of all-cause mortality and HF rehospitalization, was reached in 188 patients (40%), during a median follow-up of 325 days (IQR 85-401). Median baseline galectin-3 level was 24 ng/ml (IQR 18-34). The mean number of galectin-3 measurements available per patient was 4.3.

After adjustment for clinical factors and NT-proBNP, there was a weak association between baseline galectin-3 and risk of the primary endpoint. When repeated measurements were taken into account, the adjusted hazard ratio per 1 standard deviation increase of the galectin-3 level (on the log2 scale) at any time point increased to 1.67 (95% CI 1.24 – 2.23, p-value <0.001). After additional adjustment for repeated NT-proBNP measurements, the association remained statistically significant.

Conclusions

Repeated galectin-3 measurements appeared a strong predictor of outcome in acute HF patients, independent of NT-proBNP. Hence, galectin-3 may be helpful in clinical practice for prognostication and treatment monitoring.

Introduction

Most studies on serum biomarkers in heart failure (HF) populations conducted so far, have related adverse outcome during follow-up with a single measurement at baseline.[1-3] Although this approach has demonstrated the prognostic value of a variety of biomarkers, among which the well-known natriuretic peptides,[4] it does not explore the biological variation within patients with evolving disease. In fact, HF is a highly variable, heterogeneous and progressive condition.[5] Thus, repeated biomarker measurements may be required to more accurately reflect this dynamic and progressive nature of the underlying pathophysiologic processes, such as mechanical overload, atherosclerosis, inflammation and cardiac fibrosis. Therefore, we expect that risk models that account for repeated measurements may more adequately reflect the current status of the patient compared to models that only use single measurements.

The TRanslational Initiative on Unique and novel strategies for Management of Patients with Heart failure (TRIUMPH) study was designed to identify and validate novel biomarkers to improve prognostication in HF.[6] TRIUMPH was designed as a translational study program, combining biological discovery of novel biomarkers, technologic advances and clinical validation in patients presenting with acute HF. In the clinical validation study, both the novel and established HF biomarkers were evaluated for their prognostic properties using a unique design of seven planned repeated measurements during 1-year follow-up. Based on previous clinical and epidemiological studies, galectin-3 was earmarked as a biomarker with high potential for improving prognostication.

Galectin-3 is a member of a large family of β-galactoside-binding animal lectins.[7] Galectin-3 expression has been detected in macrophages, neutrophils, eosinophils, and mast cells. In response to a variety of mechanical and neurohormonal stimuli, macrophages secrete galectin-3.[8] Galectin-3 stimulates additional macrophages, pericytes, myofibroblasts and fibroblasts, which are all involved in the initiation and progression of tissue scarring. Consequently, galectin-3 appears to be involved in cardiac fibrosis. In addition, galectin-3 plays an important role in the inflammatory response, which is an important step in the process of cardiac remodeling.[9-11] Galectin-3 is expressed in numerous tissues such as heart, kidney, lung, uterus and colon.[12] The level of galectin-3 expression is relatively low in heart tissue under normal conditions, but may increase substantially under pathophysiological circumstances.[13]

Several clinical studies have evaluated the prognostic value of galectin-3. Higher levels of galectin-3 have been associated with an increased risk of incident HF and all-cause

mortality in the general population.[14,15] Furthermore, single galectin-3 levels have shown to be an independent risk factor of mortality in both stable and acute HF patients, although it still remains uncertain whether galectin-3 confers independent prognostic information when added to NT-proBNP.[2,3,16-18] A few studies have been performed to assess the prognostic value of galectin-3 when measured multiple times. The change in galectin-3 level over time was predictive of outcome.[19-21] However, given the dynamic and progressive nature of HF, the number of galectin-3 measurements needed for adequate estimation of the true galectin-3 level is expected to be high. Therefore, in the present study, we assessed the independent association between the estimated instantaneous galectin-3 level, using frequently measured galectin-3 levels, and the incidence of all-cause mortality and HF readmission during 1-year follow-up in the 496 patients with acute HF who compose the TRIUMPH clinical cohort.

Methods

Objective and study design

TRIUMPH was designed as a translational bench-to-bedside study program encompassing the entire spectrum of biomarker discovery to clinical validation.[6] The clinical validation study was an observational prospective study enrolling patients admitted with acute HF in 14 hospitals in The Netherlands, between September 2009 and December 2013. This cohort study was designed to validate the clinical value of biomarkers successfully passing the bio-informatics and early-validation stages of TRIUMPH, and to further evaluate more established biomarkers of HF. There was a particular interest in the change in biomarker levels over time. The study was approved by the medical ethics committee at all participating centers.

Patient selection

Patients ≥18 years of age were eligible for enrollment if they were hospitalized with decompensation of known chronic HF or newly diagnosed HF. Furthermore, three other criteria had to be met: (1) natriuretic peptide levels had to be elevated to ≥3 times the upper limit of normal (ULN), (2) there had to be evidence of sustained systolic or diastolic left ventricular dysfunction, and (3) patients had to be treated with intravenous diuretics. Patients with HF precipitated by a non-cardiac condition, by severe valvular dysfunction without sustained left ventricular dysfunction, or by an acute ST-segment elevation myocardial infarction were excluded. Furthermore, patients scheduled for a coronary revascularization procedure, on a waiting list for a heart transplantation, with severe renal failure for which dialysis was needed, or with a coexistent condition with

a life expectancy <1 year could not participate. All study participants provided written informed consent.

Patient management

Patient management was at the discretion of the treating physician, and in accordance with the guidelines of the European Society of Cardiology.[22] Importantly, the biomarker data that were generated in the context of this observational study were not used for treatment decisions.

Study procedures

During hospitalization, blood samples were obtained at admission (day 1), once during days 2 to 4 and, subsequently, on the day of discharge. Afterwards, repeated blood samples were also obtained at outpatient follow-up visits, which were planned at 2 to 4 weeks, 3 months, 6 months and 9 to 12 months after discharge. The baseline blood sample was defined as the first sample obtained after inclusion, up to a maximum of 2 days after inclusion. At each visit, HF symptoms were assessed using the NYHA classification. Medication use was determined at discharge using three categories: (1) use of an angiotensin converting enzyme inhibitor (ACE-I) or an angiotensin II receptor antagonist (ARB) or both, (2) use of a beta-blocker, (3) use of diuretics. Patients underwent physical examination and systematic measurements of weight, blood pressure and heart rate.

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. The collected material was centrifuged at 1700 G / Relative Centrifugal Force, whereafter citrate-, EDTA-, heparine-, and trasylol-plasma was separated, as well as blood serum. Buffy coats were collected from EDTA tubes to enable analysis of genetic factors. Dimethylsulfoxide (DMSO) was added to an additional EDTA tube for cryopreservation of blood cells. All blood aliquots were subsequently stored at a temperature of -80°C within 2 hours after venipuncture.

Galectin-3 measurements

Serum and heparin-plasma was transported under controlled conditions to a central laboratory (Future Diagnostics Solutions B.V.) for batch analysis of galectin-3 and NT-proBNP levels. Galectin-3 concentrations were determined in serum, using the BGM Galectin-3 Test as instructed by the manufacturer (BG Medicine, Inc, Waltham, USA). NT-proBNP concentrations were determined in heparin plasma using the Elecsys

NT-proBNP assay on a Cobas 8000 analyzer (Roche Diagnostics Limited, Rotkreuz, Switzerland). Analysts were blinded for patient characteristics and endpoints.

Endpoints

Information on vital status and hospital readmissions was obtained until at least 9 months with a maximum of 400 days after the index hospitalization. We approached the civil registry, screened all medical records, and asked patients for information during their follow-up visits.

The primary endpoint is the composite of all-cause mortality and readmission for HF. Readmission for HF was defined as an unplanned rehospitalization due to decompensation of HF, with at least two of the following three criteria being present: elevated natriuretic peptide levels ≥3 times the ULN, symptoms of cardiac decompensation (rales, edema or elevated central venous pressure), and treatment with intravenous diuretics. Secondary endpoints included the individual components of the primary endpoint and cardiovascular mortality. An event adjudication committee, blinded for biomarker information, was established for reviewing and adjudication of endpoints.

Statistical analysis

The distributions of continuous variables, including biomarker levels, were evaluated for normality by visual examination of the histogram and Kolmogorov-Smirnov tests. Variables with a normal distribution are presented as mean ± standard deviation (SD), whereas the median and interquartile range (IQR) are presented in case of non-normality. Categorical variables are presented as counts and percentages. Galectin-3 and NT-proBNP levels had a non-normal distribution and were therefore log-transformed for further analysis.

Patients were classified according to the quartiles of the galectin-3 distribution, and differences in baseline characteristics between these quartiles were evaluated by chi-square tests (categorical variables), analysis of variance, or Kruskal-Wallis tests, as appropriate.

We applied Cox proportional hazards models to evaluate the association of baseline galectin-3 levels with the study endpoints. Subjects were censored at the time of occurrence of the endpoint under investigation, death, and at the scheduled end of follow-up. No deviations of the proportional hazards assumption were found by inspecting log minus log plots of the survival functions. We performed univariate analyses

to obtain the crude estimates of the effect of baseline galectin-3 level (model 1), analyses that were adjusted for age and sex only (model 2), and, analyses that were additionally adjusted for systolic blood pressure, diabetes mellitus, left ventricular ejection fraction (LVEF), previous hospitalization for HF during the last 6 months, ischemic HF, body mass index, estimated glomerular filtration rate (eGFR) and baseline NT-proBNP level (model 3). The results are presented as adjusted hazard ratios (HR) per 1 SD increase of the biomarker level (on the log2 scale) with 95% confidence intervals (CI). We calculated the eGFR using the Modification of Diet in Renal Disease equation.[23]

Joint models were fitted to assess the association between estimated instantaneous biomarker levels, calculated using the repeated biomarker levels, and the specified study endpoints. A joint model combines a mixed-effects linear regression model for the serial measurements with a Cox proportional hazards model for the risk of the specified study endpoints.[24] We used cubic splines, with knots set at 1 week and 1 month after initial hospitalization. For the analyses with the repeated galectin-3 measurements, we used similar univariate and multivariate models as mentioned above (models 1, 2 and 3), except for model 3 we added medication use at discharge to the mixed-effects linear regression model. We also tested whether the instantaneous slope of the galectin-3 trajectories itself, when added to model 3, was an independent predictor. Finally, we combined the repeated measurements of galectin-3 and NT-proBNP to assess their respective independent prognostic value. Taking into account the limitations of the R packages for Joint Modeling, we were able to combine the estimated galectin-3 trajectory (using a mixed-effects linear regression model) and the estimated NT-proBNP trajectory (using a time-dependent Cox proportional hazards model) in one joint model. Since the model did not converge when we adjusted for all the covariates in model 3, baseline systolic blood pressure had to be left out in this final model (model 4). Diagnostics and sensitivity analyses were performed to evaluate the joint models. To account for the correlation structure between serial biomarker measurements collected from the same patient, we obtained the SD from the total variance of a random intercepts linear mixed model fitted on the post discharge data. The final results are presented as adjusted HR per 1 SD increase of the biomarker level (on the log2 scale) at any point in time with 95% CI.

The TRIUMPH sample size was chosen to achieve a power of 80% (1-β=0.8) to detect an odds ratio of at least 2.0 (α=0.05, 2-sided test) for a biomarker value above the 75% percentile of its distribution comparing endpoint cases with non-cases. The incidence of the primary endpoint was initially estimated at 25-30%, based on observations in historical heart failure populations. Then, 780 patients are required. During the course

of the study, based on evolving evidence, the estimated incidence was adjusted to 30-35%, and the sample size was eventually determined at 490 patients. TRIUMPH enrolled 496 patients, and 40% reached the primary endpoint.

Data on covariates were complete in 93% of patients, except for LVEF, which was complete in 78%. Single imputation was applied to account for missing values of covariates. Data is imputed using predictive mean matching for continuous variables, logistic regression for binary variables, and polytomous regression for unordered categorical data. Baseline covariates used in the full model and survival information were used in the imputation. The software used was R package MICE (https://cran.r-project.org/web/packages/mice/mice.pdf). A sensitivity analyses was performed on the full model for the primary end point on the complete cases.

The Statistical Package for Social Sciences, version 21.0 (SPSS, IBM corp., Armonk, NY, USA) was used for descriptive data analysis. R statistical software (version 2.15.0, available at: www.r-project.org) was used for advanced statistical analyses of the longitudinal biomarker data and study endpoints (packages JMBayes and JM). All statistical tests were two-tailed and p-values <0.05 were considered statistically significant.

Results

Patients

A total of 496 patients were enrolled in the TRIUMPH clinical cohort. 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. Their median age was 74 years (IQR 65-80) and 37% were women (Table 1). Median systolic blood pressure was 125 mmHg (IQR 110-147) and median LVEF was 30% (IQR 21-42). At discharge 78% used an ACE-I or an ARB or both, 78% used a beta-blocker and 93% used diuretics. Median baseline galectin-3 level was 24 ng/ml (IQR 18-34) and NT-proBNP 4152 pg/ml (IQR 2089-9387). Table 2 shows the baseline characteristics of patients in different quartiles of galectin-3 level. Patients in quartiles with a higher galectin-3 level were older and had a worse kidney function. In the higher galectin-3 quartiles more patients had a history of myocardial infarction and diabetes mellitus, had ischemic HF, and had been admitted to the hospital for heart failure during the last 6 months. In the lower galectin-3 quartiles more patients had newly-diagnosed HF during the initial hospitalization.

Table 1. Baseline parameters according to overall sample in study population (N=475).

Variables		Overall sample
Demographic characteristics, median (IQR) or %		
Age, years		74 (65-80)
Female		37
Caucasian		95
Measurements at baseline, median (IQR) or %		
Body mass index, kg/m2		28 (25-31)
Systolic blood pressure, mmHg		125 (110-147)
Diastolic blood pressure, mmHg		74 (65-85)
Heart rate, bpm		85 (72-100)
eGFR, ml/min/1.73m^2		46 (34-62)
Left ventricular ejection fraction, %		30 (21-41)
NYHA classification	II	17
	III	55
	IV	27
Medical history, %		
Newly diagnosed heart failure		36
Heart failure with reduced ejection fraction		83
Previous heart failure admission within 6 months		20
Ischemic heart failure		49
Myocardial infarction		40
Hypertension		51
Atrial fibrillation		42
Diabetes Mellitus		36
Stroke		17
Medication use at discharge, %		
ACE-I and/or ARB		78
Beta-blocker		78
Diuretics		93
Biomarkers, median (IQR)		
Galectin-3 (ng/ml)		24 (18-34)
NT-proBNP (pg/ml)		4152 (2089-9387)

IQR = Inter quartile range, eGFR = estimated glomerular filtration rate, ACE-I = Angiotensin converting enzyme inhibitor, ARB = Angiotensin II receptor antagonist.

Baseline Galectin-3 levels and the incidence of study endpoints

During the median follow-up of 325 days (IQR 85-401), 188 patients (40%) reached the primary composite endpoint of all-cause death (n=113) or readmission for HF (n=123). This corresponds with an incidence rate (IR) of 55.9 per 100 patient-years for the primary endpoint. In the highest quartile of baseline galectin-3, 65 patients (59%) reached the primary endpoint compared to 27 patients (24%) in the lowest quartile. The number of events in the highest quartile compared to the lowest quartile of galectin-3 was also higher for all-cause mortality (n=44 (40%) and n=14 (13%), respectively) and readmission for HF (n=44 (40%) and n=19 (17%), respectively).

Table 2. Baseline characteristics according to quartiles of galectin-3 level.

Variables		Quartile 1	Quartile 2	Quartile 3	Quartile 4	p-value*
Demographic characteristics, median or %						
Age, years		70	73	76	75	0.010
Female		45	31	33	38	0.13
Caucasian		92	93	97	96	0.27
Measurements at baseline, median (IQR) or %						
Body mass index, kg/m^2		27	27	29	29	0.035
Systolic blood pressure, mmHg		130	125	125	122	0.29
Diastolic blood pressure, mmHg		80	73	74	70	<0.001
Heart rate, bpm		94	85	84	80	0.002
eGFR, ml/min/1.73m^2		63	55	42	32	<0.001
Left ventricular ejection fraction, %		30	30	34	31	0.020
NYHA classification	II	23	18	11	14	0.12
	III	50	51	63	60	
	IV	27	28	25	26	
Medical history, %						
Newly diagnosed heart failure		57	40	26	21	<0.001
Heart failure with reduced ejection fraction		88	88	76	81	0.080
Previous heart failure admission within 6 months		8	17	24	29	<0.001
Ischemic heart failure		40	45	55	56	0.036
Myocardial infarction		28	32	54	48	<0.001
Hypertension		40	50	56	60	0.016
Atrial fibrillation		32	44	45	46	0.089
Diabetes Mellitus		20	32	41	50	<0.001
Stroke		14	14	17	22	0.29
Biomarkers, median						
NT-proBNP, pg/ml		3180	3970	4372	7544	<0.001
Galectin-3, ng/ml		16	21	28	40	<0.001

*P-value for differences between groups.
eGFR = estimated glomerular filtration rate.

Baseline galectin-3 levels were higher in patients who reached a study endpoint when compared with those who remained event-free (Figure 1). The baseline galectin-3 level was associated with an increased risk of reaching the primary endpoint, as well as with all-cause mortality, cardiovascular mortality, and HF readmission (Table 3). After adjustment for all selected potential confounders including baseline NT-proBNP level (model 3), the association between baseline galectin-3 and the different endpoints became weaker, but remained present.

Table 3. Hazard ratios for different endpoints per 1 SD increase of the *baseline* galectin-3 level (on the log2 scale).

	Mean value *			Baseline level †	
	M - SD	M	M + SD	HR (95% CI)	P-value
	15.9	24.7	38.2		
Primary endpoint					
Model 1				1.50 (1.30 - 1.75)	< 0.001
Model 2				1.49 (1.28 – 1.73)	< 0.001
Model 3				1.12 (0.93 – 1.36)	0.241
Number of events / patients		188/475			
All-cause mortality					
Model 1				1.54 (1.29 – 1.85)	< 0.001
Model 2				1.52 (1.26 – 1.83)	< 0.001
Model 3				1.26 (1.01 – 1.59)	0.044
Number of events / patients		113/475			
HF hospitalization					
Model 1				1.47 (1.22 – 1.76)	< 0.001
Model 2				1.47 (1.23 – 1.76)	< 0.001
Model 3				1.05 (0.82 – 1.33)	0.720
Number of events / patients		123/475			
Cardiovascular mortality					
Model 1				1.60 (1.28 – 1.99)	< 0.001
Model 2				1.57 (1.26 – 1.97)	< 0.001
Model 3				1.24 (0.93 – 1.67)	0.147
Number of events / patients		77/475			

***** Mean ± one standard deviation of the patient-specific geometric mean galectin-3 value at baseline (presented on the linear scale).
† Hazard ratios are related to a 1 SD increase of galectin-3 (on the log scale) at baseline.
Model 1 unadjusted; model 2 adjusted for age and sex; model 3 adjusted for age, sex, systolic blood pressure, diabetes mellitus, LVEF, previous hospitalization for heart failure during the last 6 months, ischemic heart failure, body mass index, eGFR and baseline NT-proBNP.
CI: confidence interval; HF: Heart failure; HR: hazard ratio; M: mean; SD: standard deviation.

Repeatedly measured galectin-3 levels and the incidence of study endpoints

On average, galectin-3 was available 4.3 times during follow-up. The mean galectin-3 level during follow-up was 23.8 ng/ml, an increase of 1 SD galectin-3 level on the log2 scale from the mean was 13 ng/ml. A decrease of 1 SD galectin-3 level on the log2 scale was 8 ng/ml. After adjustment for age and sex (model 2), the HR per SD increase of the galectin-3 level (on the log2 scale) at any point in time was 2.09 (95% CI 1.71 – 2.56) for the primary endpoint. After adjustment for the broader range of potential confounders including medication use at discharge and baseline NT-proBNP level (model 3), the association remained highly statistically significant with a HR of 1.67 (95% CI 1.24 – 2.23) (Table 4). Results were similar for the secondary endpoints. The instantaneous slope of the galectin-3 level trajectories itself was not an independent predictor of the primary endpoint.

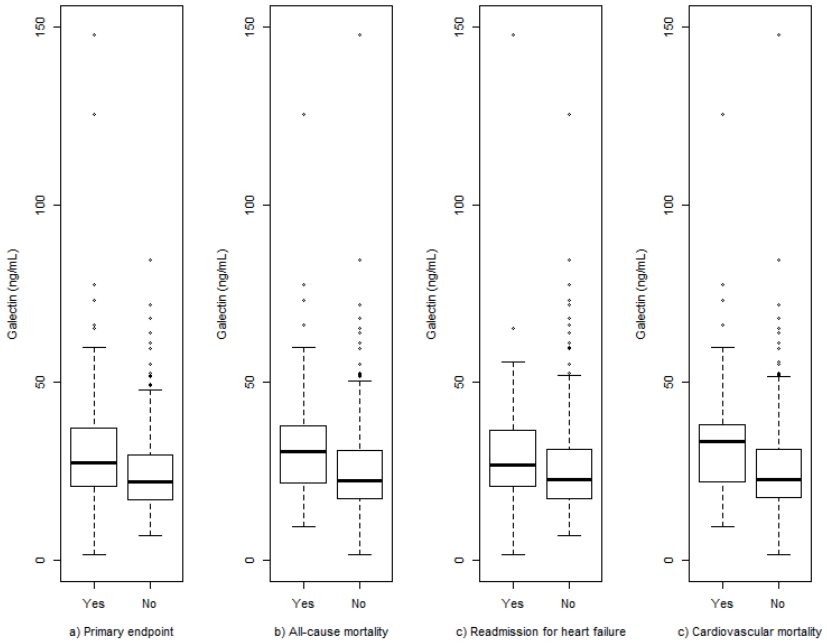

Figure 1. Distributions of baseline galectin-3 levels within the subpopulations of patients that had an event and those who did not experience an event for: a) the primary endpoint; b) the single endpoint of all-cause mortality; c) the single endpoint of readmission for heart failure; d) the single endpoint of cardiovascular mortality.

Table 4. Hazard ratios for different endpoints per 1 SD increase of the galectin-3 level (on the log2 scale) at any point in time, using a joint model.

	Mean value *			Instantaneous level †	
	M - SD	M	M + SD	HR (95% CI)	P-value
Primary endpoint	15.4	23.8	36.6		
Model 1				2.07 (1.71 – 2.53)	< 0.001
Model 2				2.09 (1.71 – 2.56)	< 0.001
Model 3				1.67 (1.24 – 2.23)	< 0.001
All-cause mortality	15.4	23.8	36.9		
Model 1				2.41 (1.83 – 3.15)	< 0.001
Model 2				2.36 (1.78 – 3.08)	< 0.001
Model 3				2.14 (1.47 – 3.16)	< 0.001
HF hospitalization	15.4	23.8	36.6		
Model 1				1.87 (1.47 – 2.39)	< 0.001
Model 2				1.92 (1.48 – 2.46)	< 0.001
Model 3				1.41 (1.02 – 1.93)	0.035
Cardiovascular mortality	15.4	23.8	36.9		
Model 1				2.46 (1.79 – 3.34)	< 0.001
Model 2				2.43 (1.76 – 3.35)	< 0.001
Model 3				2.22 (1.48 – 3.36)	< 0.001

* Mean ± one standard deviation of the patient-specific geometric mean galectin-3 value during follow-up (presented on the linear scale).

† Hazard ratios are related to a 1 SD increase of galectin-3 (on the log scale) at any point in time.

Model 1 unadjusted; model 2 adjusted for age and sex; model 3 adjusted for age, sex, systolic blood pressure, diabetes mellitus, LVEF, previous hospitalization for heart failure during the last 6 months, ischemic heart failure, body mass index, eGFR, medication use at hospital discharge (ACE-I and/or ARB, beta-blocker, diuretics) and baseline NT-proBNP level

CI: confidence interval; HF: Heart failure; HR: hazard ratio; M: mean; SD: standard deviation.

Table 5. Hazard ratios for different endpoints per 1SD increase of galectin-3 level or NT-proBNP level (on the log2 scale) at any point in time using repeated galectin-3 and NT-proBNP measurements in a joint model.

	Mean value *			Instantaneous level †	
	M – SD	M	M + SD	HR (95% CI)	P-value
Primary endpoint					
Galectin-3	15.4	23.8	36.6	1.54 (1.16 – 2.05)	0.003
NT-proBNP	742	2445	8062	2.10 (1.63 – 2.74)	< 0.001
All-cause mortality					
Galectin-3	15.4	23.8	36.9	1.77 (1.22 – 2.52)	< 0.001
NT-proBNP	739	2480	8321	2.68 (1.90 – 3.86)	< 0.001
HF hospitalization					
Galectin-3	15.4	23.8	36.6	1.29 (0.92 – 1.81)	0.160
NT-proBNP	742	2445	8062	1.71 (1.27 – 2.25)	< 0.001
Cardiovascular mortality					
Galectin-3	15.4	23.8	36.9	1.89 (1.25 – 2.85)	0.002
NT-proBNP	739	2480	8321	2.62 (1.70 – 4.27)	< 0.001

* Mean ± one standard deviation of the patient-specific geometric mean biomarker level during follow-up (presented on the linear scale).
† Hazard ratios are related to a 1 SD increase of biomarker level (on the log scale) at any point in time.
Model 4 adjusted for age, sex, diabetes mellitus, LVEF, previous hospitalization for heart failure during the last 6 months, ischemic heart failure, body mass index, eGFR, medication use at hospital discharge (ACE-I and/or ARB, beta-blocker, diuretics) and baseline NT-proBNP level
CI: confidence interval; HF: Heart failure; HR: hazard ratio; M: mean; SD: standard deviation.

After adjustment for repeated NT-proBNP measurements (model 4), the association between repeated galectin-3 levels and adverse outcome remained statistically significant with a HR of 1.54 (95% CI 1.16 – 2.05) for the primary endpoint corresponding with 1 SD increase of galectin-3 level (on the log2 scale) at any point in time (Table 5). The HR corresponding with a 1 SD increase of NT-proBNP level (on the log2 scale) at any point in time was 2.10 (95% CI 1.63 – 2.74) after adjustment for repeated galectin-3 levels.

Figure 2A shows the average estimated galectin-3 level in patients with and without the primary endpoint according to model 3 and the individual galectin-3 measurements. During hospitalization the average galectin-3 level remains steady for patients who remained free of the primary endpoint. For patients who reached the primary endpoint during follow-up the average estimated galectin-3 level decreased slightly after the initial hospitalization. Apparently, throughout follow-up, patients who reached the primary endpoint had, on average, higher levels than their counterparts who remained free of the primary endpoint. Furthermore, the average estimated galectin-3 levels appeared to elevate several weeks prior to the time of the primary endpoint (figure 2B).

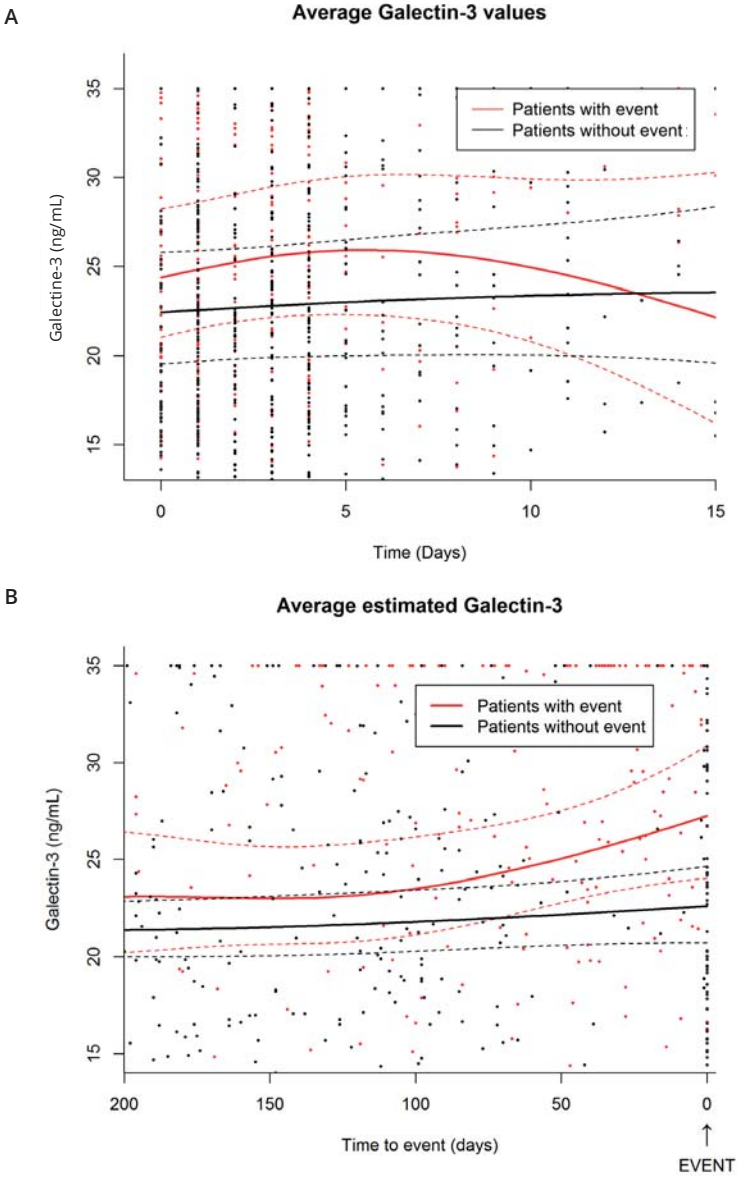

Figure 2 A. Average estimated galectin-3 pattern during initial hospitalization for decompensated heart failure for patients with and without the primary endpoint. The figure includes the individual galectin-3 measurements for patients with and without the primary endpoint.
2 B. Average estimated galectin-3 pattern prior to the primary endpoint or end of follow-up for patients with and without the primary endpoint. The figure includes the individual galectin-3 measurements for patients with and without the primary endpoint.
The average estimated galectin-3 levels are adjusted for age, sex, systolic blood pressure, diabetes mellitus, LVEF, previous hospitalization for heart failure during the last 6 months, ischemic heart failure, body mass index, eGFR, medication use at hospital discharge (ACE-I and/or ARB, beta-blocker, diuretics) and baseline NT-proBNP (model 3).

Discussion

This study clearly demonstrates that, in patients admitted with acute HF, repeated galectin-3 measurements are a strong and independent predictor of the composite endpoint of all-cause mortality or readmission for HF during 1-year follow-up. Our results illustrate that repeated measurements of galectin-3 offer incremental prognostic value to (repeatedly measured) NT-proBNP, which is considered the gold standard biomarker in HF patients.

Our observation that baseline galectin-3 level was associated with mortality confirms earlier findings both in acute and stable HF patients.[2,3,25,26] Similar to previous studies, the association between baseline galectin-3 level and mortality attenuated after adjustment for established risk factors, including kidney function and NT-proBNP level.[16, 17, 27] The association between baseline galectin-3 level and readmission for HF was less apparent. However, the decision to hospitalize a patient for decompensation of HF may be influenced by several subjective patient- and physician-related factors that are unlikely to have an association with the galectin-3 level. Furthermore, several risk factors such as kidney function, diabetes mellitus and NT-proBNP level influence this decision and are related to galectin-3. Therefore, the association between baseline galectin-3 level and heart failure readmission attenuated after adjustment for these risk factors. Since the primary endpoint is a composite of all-cause mortality and readmission for HF, the relationship between the galectin-3 level and the mortality endpoints per se are stronger compared to the primary endpoint.

Repeated galectin-3 measurements were strongly and independently related to the primary endpoint, as well as its separate components. Repeated measurements take into account the dynamic and continuous change in galectin-3 level over time, which better reflects the true nature of the underlying pathophysiology in HF. In this study, the number of galectin-3 measurements per patient was high and therefore the repeated galectin-3 measurements could be used to estimate instantaneous galectin-3 levels (i.e. the estimated galectin-3 level at any point in time during the follow-up period). When compared to baseline galectin-3 levels, the estimated instantaneous galectin-3 levels identified patients at an even higher risk for reaching an endpoint. The estimated instantaneous galectin-3 level more accurately approximates the true galectin-3 level and therefore reflects the actual condition of the patient at that point in time during follow-up. This is expected to be important since HF is a dynamic and often progressive disease in which inflammation, cardiac fibrosis and remodelling are ongoing processes that cannot be captured in a single biomarker assessment at one point in time.[5]

Furthermore, baseline galectin-3 measurements were all taken during hospitalization for decompensated chronic HF or new onset HF. It is known that galectin-3, in contrast to natriuretic peptides, does not respond to volume overload and unloading directly, which occurs during hospitalization.[28] As galectin-3 is involved in the process of myocardial fibrosis, it is more likely that galectin-3 is of more prognostic value when patients enter a more chronic phase of HF.[11]

Interestingly, the slope of the galectin-3 trajectory did not add prognostic information to the estimated instantaneous galectin-3 level. An explanation could be that galectin-3 is helpful in identifying high-risk patients when their galectin-3 level rises above a certain threshold. The change in galectin-3 level prior to reaching this threshold is not essential for risk stratification. However, to be able to estimate whether a patient's galectin-3 level rises above the threshold, repeated measurements are required. A few studies have been conducted on the prognostic value of multiple galectin-3 measurements in acute and stable HF patients.[19,20] These studies showed that change in galectin-3 level is associated with mortality. A possible explanation as to why in the present study slope of the galectin-3 trajectory did not add further prognostic information, might be that the number of galectin-3 measurements during follow-up was substantially higher in our study which allowed us to estimate an instantaneous slope of the galectin-3 trajectory, rather than the slope of the difference ('delta') between the level at baseline and that at a fixed point in time.

The statistical method (Joint Model) used to estimate the trajectory of the galectin-3 level takes into account the continuous changes in biomarker levels and adequately analyses the relation between these biomarker trajectories and different endpoints considering the changing population due to censoring at the time of occurrence of an endpoint. Previous studies presented changes in biomarker level as a 'delta' between just two measurements that are separated in time. If more than two samples are taken into account, patients have often been categorized according to the number of high or low biomarker levels. Obviously, both approaches do not fully capture the true biomarker pattern of the dynamic disease. Additionally, the power to predict adverse outcome is reduced.

An important finding of the present study is that repeated galectin-3 measurements conferred additional and independent prognostic information to that offered by baseline as well as repeated NT-proBNP measurements. The fact that NT-proBNP and galectin-3 reflect different underlying pathophysiological processes in HF may be the most important reason for this observation. Galectin-3 is a marker of cardiac fibrosis,

inflammation and remodelling, whereas NT-proBNP is a marker of volume overload.[13,29] As such, galectin-3 might be a marker that more directly reflects the pathophysiological processes that lead to adverse cardiac remodelling and deterioration of cardiac function, whereas NT-proBNP reflects the volume overload resulting from the actual (left) ventricular dysfunction. In this way, the galectin-3 and NT-proBNP level provide complimentary information on the pathophysiological state, as well as with respect to the assessment of prognosis. With respect to prognostication in HF, the results of the present study, therefore, not only provide evidence for the use of repeated galectin-3 measurements, but also for the combined use with (repeatedly-measured) NT-proBNP.

Although this study is a large multi-centre prospective observational study, it seems that the studied population is not completely representable for the average HF population. The mean age in our study population is 74 and the women are underrepresented. Moreover only 18% of the included HF patients have a preserved ejection fraction. De Boer et al.[30] showed that galectin-3 levels did not differ between HF patients with a reduced and preserved ejection fraction and the predictive value of galectin-3 was stronger in patients with a preserved ejection fraction. By underrepresenting the HF patients with a preserved ejection fraction in our study we possibly underestimated the prognostic value of galectin-3.

Future studies should evaluate the value of repeated galectin-3 measurements when used to guide treatment decisions. It may be hypothesized that treatment is to be intensified in patients with high galectin-3 levels or unfavourable galectin-3 patterns. On the other hand, repeated galectin-3 measurements might be helpful to identify patients who are more likely to respond to certain treatments.[31] Furthermore, it remains to be addressed whether galectin-3 may be targeted by specific antigalectin-3 therapies. Additional studies should also determine the number of galectin-3 measurements needed for optimal prognostication and therapy monitoring. The frequency by which galectin-3 levels should be measured may not be identical for each patient, but depends on the clinical condition of the patient, the treatment given, the galectin-3 level and the progression of galectin-3 levels during follow-up.

Conclusions

The TRIUMPH study clearly demonstrates that repeated measurements of galectin-3 are a strong and independent predictor of adverse outcome in patients following admission for acute HF. The estimated instantaneous galectin-3 level identified patients

at a higher risk of reaching adverse events than baseline galectin-3 levels alone. In addition, repeated galectin-3 measurements offer incremental prognostic value to that conferred by other known risk factors and, importantly, repeated measurements of NT-proBNP. These results suggest that repeated galectin-3 measurements in addition to NT-proBNP measurements may be helpful in clinical practice to identify HF patients who are at increased risk of adverse outcome.

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Contributors

List of Site Investigators: Hans L. Hillege, MD, PhD (UMCG Groningen, the Netherlands); Ivonne Lesman-Leegte, PhD (UMCG Groningen, the Netherlands); Michiel J. Nagelsmit, MD, PhD (Treant Zorggroep Scheperziekenhuis Emmen, the Netherlands); Elly M.C.J. Wajon, MD (Medisch Spectrum Twente Enschede, the Netherlands); Hans-Peter Brunner-La Rocca, MD, PhD (Maastricht UMC, the Netherlands); Gerard C.M. Linssen, MD, PhD (Twenteborg ziekenhuis Almelo, the Netherlands); Willem F. Terpstra, MD, PhD (Slingeland Ziekenhuis Doetinchem, the Netherlands); K. Martijn Akkerhuis, MD, PhD (Erasmus MC Rotterdam, the Netherlands); Laura C. van Vark, MD (Erasmus MC Rotterdam, the Netherlands); Eric Boersma, PhD (Erasmus MC Rotterdam, the Netherlands); Anho H. Liem, MD, PhD (Admiraal de Ruyter Ziekenhuis Goes, the Netherlands); Alexander J. Wardeh, MD, PhD (Haaglanden MC Den Haag, the Netherlands); Addy J.M. van Miltenburg, MD, PhD (Fransiscus Gasthuis & Vlietland Rotterdam, the Netherlands); Ewout J. van den Bos, MD, PhD (Albert Schweitzer Ziekenhuis Dordrecht, the Netherlands); Stijn P.J. de Ridder (St. Anna Ziekenhuis Geldrop, the Netherlands); Folkert W. Asselbergs, MD, PhD (UMC Utrecht, the Netherlands); Yigal M. Pinto, MD, PhD (AMC Amsterdam, the Netherlands).

Disclosures

None.

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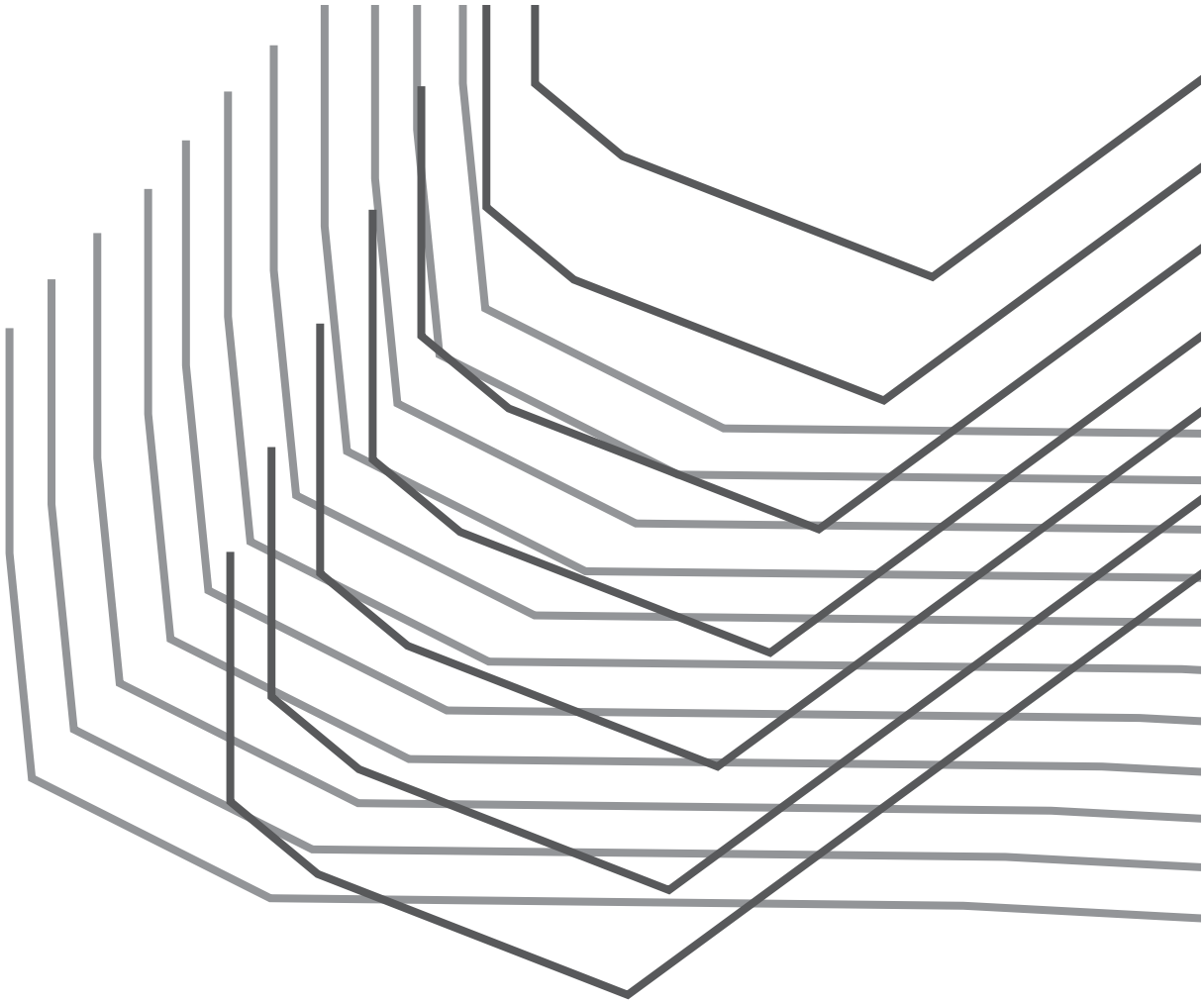

Chapter 3

Prognostic value of serial ST2 measurements in patients with acute heart failure

L.C. van Vark, I. Lesman-Leegte, S.J. Baart, D. Postmus, Y.M. Pinto, J.G. Orsel, B.D. Westenbrink, H.P. Brunner-la Rocca, A.J.M. van Miltenburg, E. Boersma, H.L. Hillege, K.M. Akkerhuis; TRIUMPH Investigators

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Abstract

Background

Several clinical studies have evaluated the association between ST2 and outcome in patients with heart failure (HF). However, only little is known about the predictive value of frequently measured ST2 levels in an acute HF population.

Objectives

To describe the prognostic value of baseline and repeated ST2 measurements in acute HF patients.

Methods

In the TRIUMPH clinical cohort study, 496 acute HF patients were enrolled in 14 hospitals in The Netherlands, between 2009 and 2014. Repeated blood samples (seven) were drawn during 1-year follow-up. ST2 and NT-proBNP levels were measured in a central laboratory. The primary endpoint was the composite of all-cause mortality and HF rehospitalization. Associations between repeated biomarker measurements and the primary endpoint were assessed using a joint model.

Results

Median age was 74 years and 37% were women. The primary endpoint was reached in 188 patients (40%), during a median follow-up of 325 days (IQR 85-401). Median baseline ST2 level was 71 ng/ml (IQR 46-102).

After adjustment for clinical factors and NT-proBNP, baseline ST2 was associated with an increased risk of the primary endpoint, hazard ratio (HR) per 1 SD increase of the baseline ST2 level (on the log2 scale) was 1.30 (95% CI 1.08 – 1.56, p-value 0.005). When repeated measurements were taken into account, the adjusted HR per 1SD increase of the ST2 level (on the log2 scale) during follow-up increased to 1.85 (95% CI 1.02 – 3.33, p-value 0.044), adjusted for clinical factors and *repeated* measurements of NT-proBNP. Furthermore, ST2 levels appear to elevate several weeks prior to the time of the primary endpoint.

Conclusions

Repeated ST2 measurements appeared a strong predictor of outcome in acute HF patients, independent of repeatedly measured NT-proBNP. Hence, ST2 may be helpful in clinical practice for prognostication and treatment monitoring.

Introduction

Heart failure (HF) is a major cause of morbidity and mortality in the Western World.[1] Improvements in treatment and patient management are needed, because most HF patients die despite evidence-based treatment. Serum biomarkers may play an important role in bridging the gap between the assessment of HF and the occurrence of adverse outcomes, and may expose novel, potentially modifiable disease pathways.

Most studies on the prognostic value of biomarkers of HF conducted so far, have related adverse outcome during follow-up with a single measurement at baseline.[2-4] This approach does not explore the biological variation that exists within patients with a highly variable, heterogeneous and progressive condition, such as HF.[5] Thus, repeated biomarker measurements may be required to more accurately reflect the dynamic and progressive nature of the underlying pathophysiologic processes, such as mechanical overload, cardiac fibrosis and inflammation and therefore may be more suitable for prognostication and therapy monitoring.

ST2 is an IL-1 receptor family member with membrane-bound (ST2L) and soluble (sST2) isoforms. An IL-1 related protein, called interleukin-33, was identified as a functional ligand for ST2L.[6] IL-33/ST2L signalling protects the myocardium against hypertrophy and cardiac fibrosis following pressure overload.[7] Soluble ST2, which is the form measured by current assays, acts as a decoy receptor for IL-33 and prevents the IL-33/ST2L interaction and the subsequent cardioprotective cascade of events. The major source of ST2 is currently not fully established. For a long time, the source of circulating sST2 in cardiac disease was presumed to be myocardial, following in vitro data that sST2 has been shown to be secreted by cardiomyocytes when the cells are subjected to biomechanical overload.[8] Accordingly serum ST2 levels correlate strongly with serum levels of natriuretic peptides.[9] More recent work, however, suggests that in human cardiac disease, the vascular endothelial cells might be the predominant source of sST2, rather than the human myocardium.[10]

In clinical studies, single ST2 levels have shown to be a risk factor of mortality in both stable and acute HF patients, independent of NT-proBNP.[2,11,12] A recent meta-analysis supports the use of ST2 in stable chronic HF patients for risk stratification.[12] Furthermore, a number of studies have evaluated the prognostic value of multiple ST2 measurements.[9,13-15] It is known that ST2 levels in acute HF patients are significantly higher than in chronic HF patients and fall rapidly over days to weeks during HF treatment.[13] This lack of reduction in ST2 level during acute HF treatment is predictive of mortality. Also, persistently high

levels of ST2 were associated with increased mortality risk.[16] Only a few studies, most in chronic systolic HF populations, have evaluated the prognostic value of the change in ST2 levels, in which the ST2 level was measured with an interval of at least 1 month.[14,15] Increases in ST2 levels, from baseline to 12 months, were associated with a significant increased risk for all-cause mortality. On the contrary, the CORONA study showed that change in ST2 levels, from baseline to 3 months, was not associated with mortality.[17] The RELAX-AHF trial showed that serial sST2 measurements combined in a multimarker approach are useful for prognostication in acute HF patients.[18]

Given the dynamic and progressive nature of HF and the pathophysiology of ST2, we hypothesized that in patients admitted with acute HF, frequently measured ST2 levels during follow-up will add incremental prognostic information to that conferred by repeated measurements of NT-proBNP. In the AHA/ACC guidelines for management of heart failure, ST2 is considered useful for prognostication and therapy monitoring, but more research is required to support this suggestion.[19] Therefore, in the present TRIUMPH study, we assessed the association between frequently measured ST2 independent of frequently measured NT-proBNP and the incidence of all-cause mortality and HF readmission during 1-year follow-up in 496 patients admitted with acute HF.

Methods

Objective and study design

TRIUMPH was designed as a translational bench-to-bedside study program encompassing the entire spectrum of biomarker discovery to clinical validation.[20] The clinical validation study was an observational prospective study enrolling patients admitted with acute HF in 14 hospitals in The Netherlands, between September 2009 and December 2013. This cohort study was designed to validate the clinical value of biomarkers successfully passing the bio-informatics and early-validation stages of TRIUMPH, as well as further evaluate more established biomarkers of HF. There was a particular interest in the change in biomarker levels over time, as well as in the analyses and prognostic significance of repeated biomarker sampling during the follow-up of HF patients. The study was approved by the medical ethics committee at all participating centres.

Patient selection

Patients ≥18 years of age were eligible for enrolment if they were hospitalized with decompensation of known chronic HF or newly diagnosed HF. Furthermore, three

other criteria had to be met: (1) natriuretic peptide levels had to be elevated to ≥3 times the upper limit of normal, (2) there had to be evidence of sustained systolic or diastolic left ventricular dysfunction, and (3) patients had to be treated with intravenous diuretics. Patients with HF precipitated by a non-cardiac condition, by severe valvular dysfunction without sustained left ventricular dysfunction, or by an acute ST-segment elevation myocardial infarction were excluded. Furthermore, patients scheduled for a coronary revascularization procedure, on a waiting list for a heart transplantation, with severe renal failure for which dialyses was needed, or with a coexistent condition with a life expectancy <1 year could not participate. All study participants provided written informed consent.

Patient management

Patient management was at the discretion of the treating physician, and in accordance with the guidelines of the European Society of Cardiology.[21] Importantly, the biomarker data that were generated in the context of this observational study were not used for treatment decisions.

Study procedures

During hospitalization, blood samples were obtained at admission (day 1), once during days 2 to 4 and, subsequently, on the day of discharge. Afterwards, repeated blood samples were also obtained at outpatient follow-up visits, which were planned at 2 to 4 weeks, 3 months, 6 months and 9 to 12 months after discharge. The baseline blood sample was defined as the first sample obtained after inclusion, up to a maximum of 2 days after inclusion. At each visit, HF symptoms were assessed using the NYHA classification. Medication use was determined at discharge using three categories: (1) use of an angiotensin converting enzyme inhibitor (ACE-I) or an angiotensin II receptor antagonist (ARB) or both, (2) use of a beta-blocker, (3) use of diuretics. Patients underwent physical examination, and weight, blood pressure and heart rate were systematically measured.

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. The collected material was centrifuged at 1700 G / Relative Centrifugal Force, whereafter heparine-plasma and blood serum were separated. All blood aliquots were subsequently stored at a temperature of -80°C within 2 hours after venipuncture.

ST2 measurements

Serum and heparin-plasma samples were transported under controlled conditions to a central laboratory (Future Diagnostics Solutions B.V.) for batch analysis of ST2 and NT-proBNP levels. ST2 concentrations were determined in serum in single measurements using a quantitative sandwich monoclonal enzyme-linked immunosorbent assay (Presage® ST2 Assay; Critical Diagnostics, Inc, San Diego, USA). In our hands the average coefficient of variation (CV) for inter-assay variation was 4.9%, in line with the average inter-assay CV of 5.2% reported by the manufacturer. NT-proBNP concentrations were determined in heparin plasma using the Elecsys NT-proBNP electrochemiluminescent sandwich immunoassay on a Cobas 8000 analyzer (Roche Diagnostics Limited, Rotkreuz, Switzerland). Analysts were blinded for patient characteristics and endpoints.

ST2 pattern

Post hoc analyses were performed to identify ST2 patterns in patients with and without the primary endpoint. Two investigators, blinded for baseline patient characteristics and clinical outcomes data, individually analysed the ST2 pattern. ST2 patterns were (1) "U-shaped" if the ST2 level initially decreased and afterwards increased, (2) "J-shaped" if the ST2 level initially decreased and did not increase afterwards, (3) "not interpretable" if less than three ST2 measurements were available or three ST2 measurements were close together, finally ST2 patterns were classified as (4) "other" if a different ST2 pattern was identified. If there was disagreement, a consensus was reached in a separate session.

Endpoints

Information on vital status and hospital readmissions was obtained until at least 9 months with a maximum of 400 days after the index hospitalization. We approached the civil registry, screened all medical records, and asked patients for information during their follow-up visits.

The primary endpoint is the composite of all-cause mortality and readmission for HF. Readmission for HF was defined as an unplanned rehospitalization due to decompensation of HF, with at least two of the following three criteria being present: elevated natriuretic peptide levels ≥3 times the upper limit of normal, symptoms of cardiac decompensation (rales, oedema or elevated central venous pressure), and treatment with intravenous diuretics. Secondary endpoints included the individual components of the primary endpoint and cardiovascular mortality. An event adjudication committee, blinded for biomarker information, was established for reviewing and adjudication of endpoints.

Statistical analysis

The distributions of continuous variables were evaluated for normality by visual examination of the histogram and Kolmogorov-Smirnov tests. Variables with a normal distribution are presented as mean ± standard deviation (SD), whereas the median and interquartile range (IQR) are presented in case of non-normality. Categorical variables are presented as counts and percentages. ST2 and NT-proBNP levels had a non-normal distribution and were therefore log-transformed for further analyses.

Patients were classified according to the quartiles of the ST2 distribution, and differences in baseline characteristics between these quartiles were evaluated by chi-square tests (categorical variables), analysis of variance, or Kruskal-Wallis tests, as appropriate.

We applied Cox proportional hazards models to evaluate the association of baseline ST2 levels with the study endpoints. Subjects were censored at the time of occurrence of the endpoint under investigation, death, and at the scheduled end of follow-up. No deviations of the proportional hazards assumption were found by inspecting log minus log plots of the survival functions. We performed univariate analyses to obtain the crude estimates of the effect of baseline ST2 level (model 1), analyses that were adjusted for age and sex only (model 2), and analyses that were additionally adjusted for systolic blood pressure, diabetes mellitus, left ventricular ejection fraction (LVEF), previous hospitalization for HF during the last 6 months, ischemic HF, body mass index, estimated glomerular filtration rate (eGFR) and baseline NT-proBNP level (model 3). The results are presented as adjusted hazard ratios (HR) per 1SD increase of the biomarker level (on the log2 scale) with 95% confidence intervals (CI). We calculated the eGFR using the Modification of Diet in Renal Disease equation.[22]

Joint models were fitted to assess the association between estimated instantaneous biomarker levels during follow-up, calculated using the repeated time-dependent biomarker levels, and the specified study endpoints. A joint model combines a mixed-effects linear regression model for the serial measurements with a Cox proportional hazards model for the risk of the specified study endpoints.[23] We used cubic splines, with knots set at 1 week and 1 month after initial hospitalization, for the mixed model. For the analyses with the *repeated* ST2 measurements, we performed univariate analyses (model 1). We combined repeated measurements of ST2 and NT-proBNP in one joint model to assess their independent prognostic value, and adjusted for age and sex (model 2). We additionally adjusted for systolic blood pressure, diabetes mellitus, LVEF, previous hospitalization for HF during the last 6 months, ischemic HF, body mass index, eGFR and use of medication at hospital discharge (ACE-I and/or ARB, beta-blocker,

diuretics) (model 3). We also tested whether the slope of the ST2 trajectories itself, when added to model 3, was an independent predictor. Diagnostics and sensitivity analyses were performed to evaluate the joint models. The final results are presented as adjusted HR per 1 SD increase of the biomarker level (on the log2 scale) at any point in time with 95% CI.

Data on covariates were complete in 93% of patients, except for LVEF, which was complete in 78%. Single imputation was applied to account for missing values of covariates.

The Statistical Package for Social Sciences, version 21.0 (SPSS, IBM corp., Armonk, NY, USA) was used for descriptive data analysis. R statistical software (version 2.15.0, available at: www.r-project.org) was used for advanced statistical analyses of the longitudinal biomarker data and study endpoints (packages JMBayes and JM). All statistical tests were two-tailed and p-values <0.05 were considered statistically significant.

Results

Patients

A total of 496 patients were enrolled in the TRIUMPH clinical cohort. 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. Their median age was 74 years (IQR 65-80) and 37% were women (Table 1). Median systolic blood pressure was 125 mmHg (IQR 110-147) and median LVEF was 30% (IQR 21-42). The majority were HF patients with a reduced ejection fraction (83%). Median baseline ST2 level was 71 ng/ml (IQR 46-102) and NT-proBNP 4152 pg/ml (IQR 2089-9387). Additionally, table 1 shows the baseline characteristics of patients in different quartiles of ST2 level. Patients in quartiles with a higher ST2 level had a worse kidney function and more patients had a history of myocardial infarction.

Table 1. Baseline characteristics according to the overall sample (n=475) and quartiles of *baseline* ST2 level (n=386)

Variables	Overall sample	Q 1	Q 2	Q 3	Q 4	p-value*
Demographic characteristics, median (IQR)or %						
Age, years	74 (65-80)	72	75	73	74	0.427
Female	37	45	37	38	34	0.434
Caucasian	95	91	95	95	95	0.541
Measurements at baseline, median (IQR) or %						
Body mass index, kg/m^2	28 (25-31)	28	28	28	27	0.768
Systolic blood pressure, mmHg	125 (110-147)	128	135	124	124	0.534
Diastolic blood pressure, mmHg	74 (65-85)	75	76	72	74	0.513
Heart rate, bpm	85 (72-100)	85	86	84	84	0.503
eGFR, ml/min/1.73m^2	46 (34-62)	51	49	44	40	0.002
Left ventricular ejection fraction, %	30 (21-41)	34	30	30	29	0.204
NYHA classification II	17	20	16	16	11	
III	55	53	58	63	53	0.378
IV	27	27	25	20	34	
Medical history, %						
Newly diagnosed heart failure	36	43	40	37	27	0.088
Heart failure with reduced ejection fraction	83	78	85	79	87	0.434
Previous heart failure admission within 6 months	20	20	18	15	27	0.245
Ischemic heart failure	49	43	44	47	53	0.498
Myocardial infarction	40	35	31	43	50	0.034
Hypertension	51	55	55	46	48	0.470
Atrial fibrillation	42	38	45	43	46	0.640
Diabetes Mellitus	36	32	32	41	39	0.439
Stroke	17	13	16	16	19	0.718
Biomarkers, median (IQR)						
ST2, ng/ml	71 (46-102)	37	59	89	132	
NT-proBNP, pg/ml	4152 (2089-9387)	2347	3970	4871	5692	<0.001
Endpoints, %						
Primary endpoint	40	23	34	44	52	<0.001
All-cause mortality	24	7	20	26	32	<0.001
HF Hospitalization	26	20	27	33	34	0.15
Cardiovascular mortality	16	2	15	17	23	<0.001

*p-value for differences between quartiles of *baseline* ST2 level.
Q: quartile; IQR: Inter quartile range; eGFR: estimated glomerular filtration rate; NYHA: New York Heart Association; HF: Heart Failure.

Baseline ST2 levels and the incidence of study endpoints

During the median follow-up of 325 days (IQR 85-401), 188 patients (40%) reached the primary endpoint of all-cause death (n=113) or readmission for HF (n=123). This corresponds with an incidence rate (IR) of 55.9 per 100 patient-years for the primary endpoint. Baseline ST2 levels were available in 386 patients. In the highest quartile of baseline ST2, 50 patients (52%) reached the primary endpoint compared to 22 patients (23%) in the lowest quartile of ST2. All-cause mortality was also higher in the highest ST2

quartile compared to the lowest ST2 quartile: 31 (32%) and 7 (7%), respectively. This was similar for cardiovascular mortality 22 (23%) and 2 (2%) respectively (Table 1).

The baseline ST2 level was associated with an increased risk of all of the predefined study endpoints (Table 2). With respect to the primary endpoint, all-cause mortality and cardiovascular mortality, these associations remained statistically significant after adjustment for all selected potential confounders including baseline NT-proBNP level (model 3).

Table 2. Hazard ratios for different endpoints per 1 SD increase of the *baseline* ST2 level (on the log2 scale).

	Mean value *			Baseline level †	
	M - SD 40.7	M 70	M + SD 120.3	HR (95% CI)	P-value
Primary endpoint					
Model 1				1.49 (1.26 – 1.77)	< 0.001
Model 2				1.48 (1.25 – 1.76)	< 0.001
Model 3				1.30 (1.08 – 1.56)	0.005
Number of events / patients		188/475			
All-cause mortality					
Model 1				1.80 (1.41 – 2.29)	< 0.001
Model 2				1.77 (1.39 – 2.27)	< 0.001
Model 3				1.43 (1.11 – 1.86)	0.006
Number of events / patients		113/475			
HF hospitalization					
Model 1				1.33 (1.09 – 1.61)	0.005
Model 2				1.33 (1.09 – 1.61)	0.005
Model 3				1.16 (0.94 – 1.43)	0.159
Number of events / patients		123/475			
Cardiovascular mortality					
Model 1				2.01 (1.49 – 2.72)	< 0.001
Model 2				1.98 (1.46 – 2.67)	< 0.001
Model 3				1.63 (1.19 – 2.23)	0.002
Number of events / patients		77/475			

* Mean ± one standard deviation of the patient-specific geometric mean ST2 value at baseline (presented on the linear scale).

† Hazard ratios are related to a 1 SD increase of ST2 (on the log scale) at baseline.

Model 1 unadjusted; model 2 adjusted for age and sex; model 3 adjusted for age, sex, systolic blood pressure, diabetes mellitus, LVEF, previous hospitalization for heart failure during the last 6 months, ischemic heart failure, body mass index, eGFR and baseline NT-proBNP.

CI: confidence interval; HF: Heart failure; HR: hazard ratio; M: mean; SD: standard deviation.

Prognostic value of repeated ST2 measurements

The average number of ST2 measurements per patient during follow-up was 3.9 and 4.1 for NT-pro-BNP. After adjustment for repeated measurements of NT-pro-BNP, age and sex (model 2), the HR for the primary endpoint corresponding with 1 SD increase of ST2

level (on the log2 scale) during follow-up was 3.54 (95% CI 2.07 – 7.32, *p-value < 0.001*). After adjustment for the broader range of potential confounders including repeated measurements of NT-proBNP (model 3), the association remained statistically significant with a HR corresponding with 1 SD increase of ST2 level (on the log2 scale) during follow-up of 1.85 (95% CI 1.02 – 3.33, *p-value 0.044*). The HR corresponding with 1 SD increase of NT-proBNP level (on the log2 scale) during follow-up for the primary endpoint was 2.13 (95% CI 1.35-3.88, *p-value < 0.001*) adjusted for model 3 and repeated measurements of ST2 (Table 3). The HRs for all-cause and cardiovascular mortality corresponding with 1 SD increase of ST2 level (on the log2 scale) during follow-up after adjustment for all covariates and repeated measurements of NT-proBNP (model 3) were highly statistically significant: 4.36 (95% CI 2.31 – 8.92, *p-value < 0.001*) and 3.98 (95% CI 2.15 – 7.94, *p-value < 0.001*), respectively. The slope of the ST2 level trajectories itself was not an independent predictor of the primary endpoint.

Figure 1 shows the measured ST2 levels of 3 individuals who showed a "U-shaped" ST2 pattern and of 3 individuals who showed a "J-shaped" pattern. 56% of the patients who reached the primary endpoint had a "U-shaped" ST2 pattern preceding the occurrence of the endpoint event, as illustrated in figure 1 patient I, II and III. Figure 1 patient IV, V and VI are examples of "J-shaped" ST2 patterns in patients who did not reach the primary endpoint. When a "J-shaped" ST2 pattern was present during follow-up, 82% of the patients remained event free.

Figure 2 shows the average estimated biomarker level and the individual biomarker measurements in patients with and without the primary endpoint adjusted according to model 3. During initial hospitalization, when all patients were treated for decompensated HF, the average estimated ST2 level decreased (Figure 2A). Following initial hospitalization, the average estimated ST2 level in patients who reached the primary endpoint were higher than their counterparts who remained primary endpoint-free. Furthermore, the average estimated ST2 levels increased several weeks prior to the time of the primary endpoint (Figure 2B). The shape of the average estimated NT-proBNP pattern following initial hospitalization, is comparable to that of the average estimated ST2 pattern (Figure 2C).

Table 3. Hazard ratios for different endpoints per 1SD increase of ST2 level or NT-proBNP level (on the log2 scale) at any point in time using repeated ST2 and repeated NT-proBNP measurements in a joint model.

	Model	Mean value *			Instantaneous level †	
		M - SD	M	M + SD	HR (95% CI)	P-value
Primary endpoint						
ST2 (crude)	1	24.2	41.4	70.9	2.78 (2.16 – 3.64)	< 0.001
ST2	2	24.2	41.4	70.9	3.54 (2.07 – 7.32)	< 0.001
NT-proBNP	2	517	1776	6093	1.67 (1.20 – 2.34)	0.002
ST2	3	24.2	41.4	70.9	1.85 (1.02 – 3.33)	0.044
NT-proBNP	3	517	1776	6093	2.13 (1.35 – 3.88)	< 0.001
All-cause mortality						
ST2 (crude)	1	24.8	42.6	73.3	4.45 (3.12 – 6.39)	< 0.001
ST2	2	24.8	42.6	73.3	4.19 (2.31 – 8.79)	< 0.001
NT-proBNP	2	545	1874	6447	1.85 (1.22 – 2.83)	0.002
ST2	3	24.8	42.6	73.3	4.36 (2.31 – 8.92)	< 0.001
NT-proBNP	3	545	1874	6447	2.48 (1.35 – 6.10)	0.004
HF hospitalization						
ST2 (crude)	1	24.2	41.4	70.9	2.24 (1.68 – 3.01)	< 0.001
ST2	2	24.2	41.4	70.9	1.80 (1.27 – 2.56)	< 0.001
NT-proBNP	2	517	1776	6093	1.62 (1.18 – 2.19)	< 0.001
ST2	3	24.2	41.4	70.9	1.10 (0.64 – 1.83)	0.690
NT-proBNP	3	517	1776	6093	1.47 (0.92 – 2.45)	0.096
Cardiovascular mortality						
ST2 (crude)	1	24.8	42.6	73.3	5.27 (3.31 – 8.31)	< 0.001
ST2	2	24.8	42.6	73.3	4.55 (2.47 – 8.37)	< 0.001
NT-proBNP	2	545	1874	6447	1.66 (1.05 – 2.67)	0.022
ST2	3	24.8	42.6	73.3	3.98 (2.15 – 7.94)	< 0.001
NT-proBNP	3	545	1874	6447	1.85 (1.02 – 3.45)	0.046

* Mean ± one standard deviation of the patient-specific geometric mean biomarker level during follow-up (presented on the linear scale).

† Hazard ratios are related to a 1 SD increase of biomarker level (on the log scale) at any point in time.

Model 1 unadjusted; Model 2 adjusted for repeated measurements of NT-proBNP or ST2, age and sex; Model 3 adjusted for repeated measurements of NT-proBNP or ST2, age, sex, systolic blood pressure, diabetes mellitus, LVEF, previous hospitalization for heart failure during the last 6 months, ischemic heart failure, body mass index, eGFR and use of medication at hospital discharge (ACE-I and/or ARB, beta-blocker, diuretics).

CI: confidence interval; HF: Heart failure; HR: hazard ratio; M: mean; SD: standard deviation.

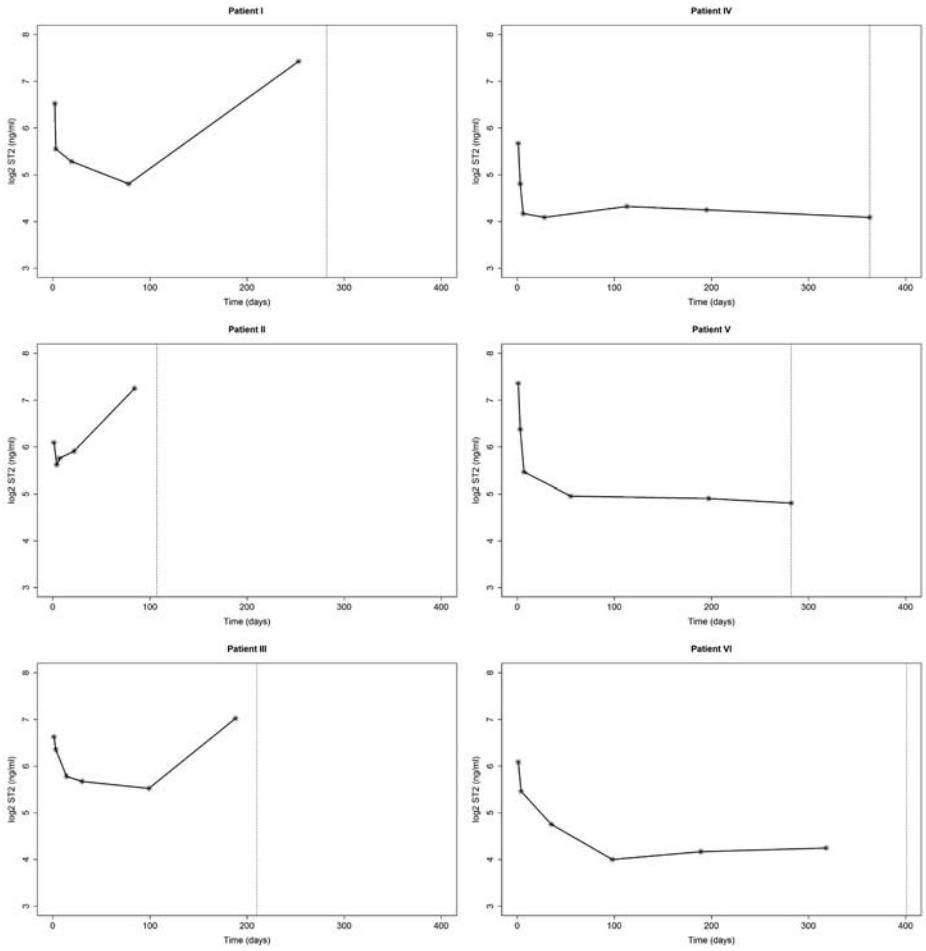

Figure 1. Examples of the ST2 pattern during follow-up in different patients.
The ST2 level of six patients during follow-up. The vertical dotted line represents the time of occurrence of the primary endpoint or the scheduled end of follow-up. Patient I, II and III demonstrate a U-shaped ST2 pattern, and reach the primary endpoint. Patient IV, V and VI demonstrate a J-shaped ST2 pattern, and remained event-free during follow-up.

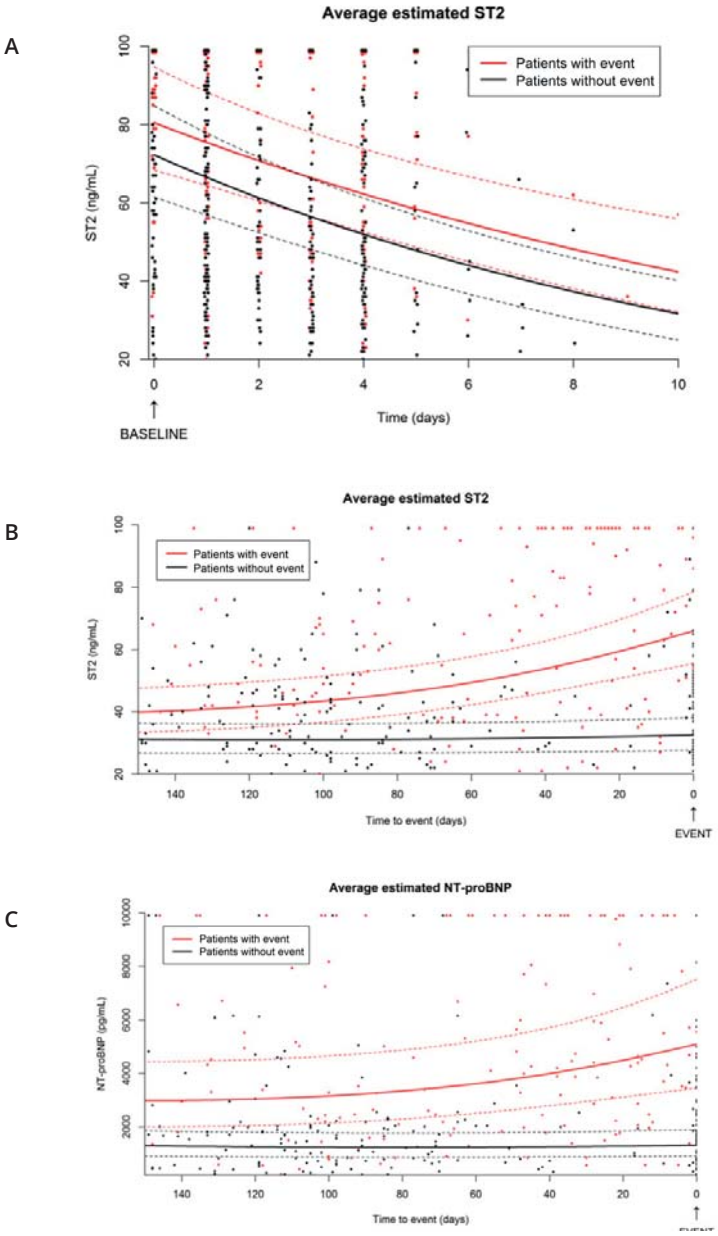

Figure 2. Average estimated biomarker pattern, combined with individual biomarker measurements during follow-up in patients with and without the primary endpoint. **A** Average estimated ST2 pattern during initial hospitalization for decompensated heart failure for patients with and without the primary endpoint. **B** Average estimated ST2 pattern prior to the primary endpoint or end of follow-up for patients with and without the primary endpoint. **C** Average estimated NT-proBNP pattern prior to the primary endpoint or end of follow-up for patients with and without the primary endpoint. The average estimated ST2 and NT-proBNP levels are adjusted for age, sex, systolic blood pressure, diabetes mellitus, LVEF, previous hospitalization for heart failure during the last 6 months, ischemic heart failure, body mass index, eGFR, use of medication at hospital discharge (ACE-I and/or ARB, beta-blocker, diuretics) (model 3).

Discussion

This study clearly demonstrates that baseline ST2 levels but even more so repeated ST2 measurements are a strong and independent predictor of the composite endpoint of all-cause mortality or readmission for HF during 1-year follow-up in patients admitted with acute HF. Our results support the concept that serial measurements of ST2 offer substantial incremental prognostic value to (repeatedly-measured) NT-proBNP, which is still considered the gold standard biomarker in HF.

The TRanslational Initiative on Unique and novel strategies for Management of Patients with Heart failure (TRIUMPH) study was designed to identify and validate novel biomarkers to improve prognostication in HF.[20] TRIUMPH was designed as a translational study program, combining biological discovery of novel biomarkers, technologic advances and clinical validation in patients presenting with acute HF. In the clinical validation study, the biomarkers were evaluated for their prognostic properties using a unique design of repeated measurements during 1-year follow-up. Within TRIUMPH, ST2 was labelled as a biomarker with high potential for improving prognostication.

It has been established that ST2 levels in acutely decompensated HF patients are useful for prognostication.[3,24,25] Our observation that baseline ST2 level was significantly associated with all of the predefined study endpoints confirms this. In line with previous studies, the association between baseline ST2 level and readmission for HF is weaker than the association between baseline ST2 and the mortality endpoints when adjusted for all potential confounders and baseline NT-proBNP.

Repeated ST2 measurements were strongly related to the primary endpoint, as well as its separate components. The association between repeated ST2 level and the primary endpoint was highly significant and considerably stronger than the association between baseline ST2 level and the primary endpoint. Repeated measurements take into account the dynamic and continuous change in ST2 level over time, which may better reflect the true changes that occur in the underlying pathophysiological processes in the individual HF patient. In this study, repeated ST2 measurements were used to estimate the instantaneous ST2 levels (i.e. the estimated ST2 level at any point in time during the follow-up period). These estimated instantaneous ST2 levels were strongly associated with the occurrence of the predefined endpoints, most likely because the level of the estimated ST2 level is close to the true ST2 level, and therefore reflects the true cardiac condition of the patient at that point in time during follow-up. This is important since HF is a dynamic and often progressive disease in which inflammation, cardiac fibrosis

and remodelling are on-going processes that cannot be captured in a single biomarker assessment at one point in time.[5]

Another finding of the present study is that the estimated average ST2 levels increase in patients prior to reaching the primary endpoint, whereas the average estimated ST2 level in patients without the primary endpoint during follow-up stabilizes. The slope of the ST2 trajectory itself did not add significant prognostic information to the estimated instantaneous ST2 level. An explanation for this finding could be that the distribution of the biomarker measurements is not ideal for assessment of the instantaneous slope. To clarify these findings, a post-hoc analysis was performed to define the ST2 pattern in individual patients. This analysis demonstrated that almost twice as many patients who reached the primary endpoint during follow-up had a so-called "U-shaped" ST2 pattern, compared to patients without an event. Furthermore, when a "J-shaped" ST2 pattern was identified, 82% of these patients remained event-free during 1 year of follow-up. Although we acknowledge that the classification of the ST2 pattern may be affected by subjectivity and that one should be careful with drawing conclusions from this post-hoc analyses, these findings suggest that the progression of the ST2 levels may be important for the evaluation of a HF patient. The increase or stabilization of the ST2 level may be a useful variable in daily practice not only for stratifying patients in high risk and low risk categories but even more so for acting on an anticipated cardiac deterioration of a patient when the ST2 levels rise during the outpatient clinic follow-up visit.

Another important finding of the present study is that repeated ST2 measurements conferred additional and independent prognostic information to that offered by repeated NT-proBNP measurements. The fact that NT-proBNP and ST2 levels reflect different underlying pathophysiological processes in HF may be the most important reason for this observation. NT-proBNP is a marker of volume overload.[26] ST2 responds to mechanical overload as well, but is also a marker of cardiac fibrosis, inflammation and remodelling.[8] In this way, ST2 and NT-proBNP levels provide complementary information on the pathophysiological state, as well as with respect to the assessment of prognosis. With respect to prognostication in HF, the results of the present study, therefore, not only provide evidence for the use of repeated ST2 measurements, but also for the combined use with (repeatedly-measured) NT-proBNP levels.

This is the first study that combines repeated ST2 measurements and repeated NT-proBNP measurements in acute HF patients and therefore adds important evidence to the statement in the AHA/ACC guidelines for management of heart failure, that ST2 is considered useful for prognostication and therapy monitoring, in addition to the use of

NT-proBNP.[19]

Future studies should evaluate the value of repeated ST2 measurements when used to guide treatment decisions. It may be hypothesized that treatment is to be intensified in patients with high ST2 levels or unfavourable (increasing) ST2 patterns. Moreover, repeated ST2 measurements may be helpful to identify patients who are more likely to respond to certain treatments. Additional studies should also determine the number of ST2 measurements needed for optimal prognostication and therapy monitoring. The frequency by which ST2 levels should be measured may not be identical for each patient, but depends on the clinical condition of the patient, the treatment given, the ST2 level and the progression of ST2 levels during follow-up. Based on these factors an individual survival curve could be plotted, which should be used for planning of the next ST2 measurement. Due to the significant lower biologic variability of ST2 compared to NT-proBNP in stable HF patients, it has been suggested that ST2 may be a better biomarker for monitoring HF patients.[27]

Conclusions

The TRIUMPH study clearly demonstrates that repeated measurements of ST2 are a strong and independent predictor of adverse outcome in patients following admission for acute HF. The repeated ST2 measurements identified patients at a substantial higher risk of reaching adverse events than baseline ST2 levels alone. In addition, repeated ST2 measurements offer incremental prognostic value to that conferred by other known risk factors and, importantly, repeated measurements of NT-proBNP. These results suggest that repeated ST2 measurements in addition to NT-proBNP measurements may be helpful in clinical practice to identify HF patients who are at increased risk of adverse outcome.

Sources of funding

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Disclosures

None.

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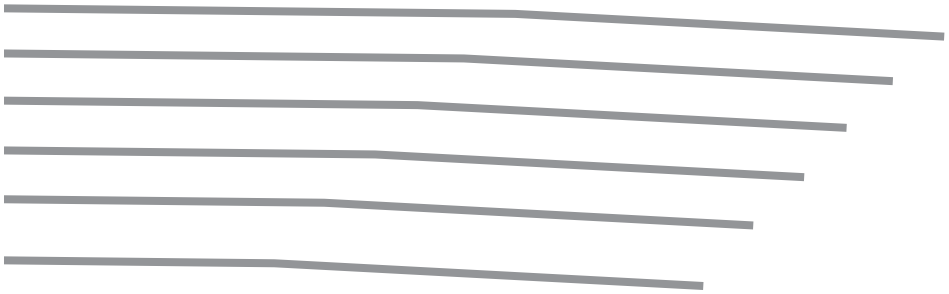

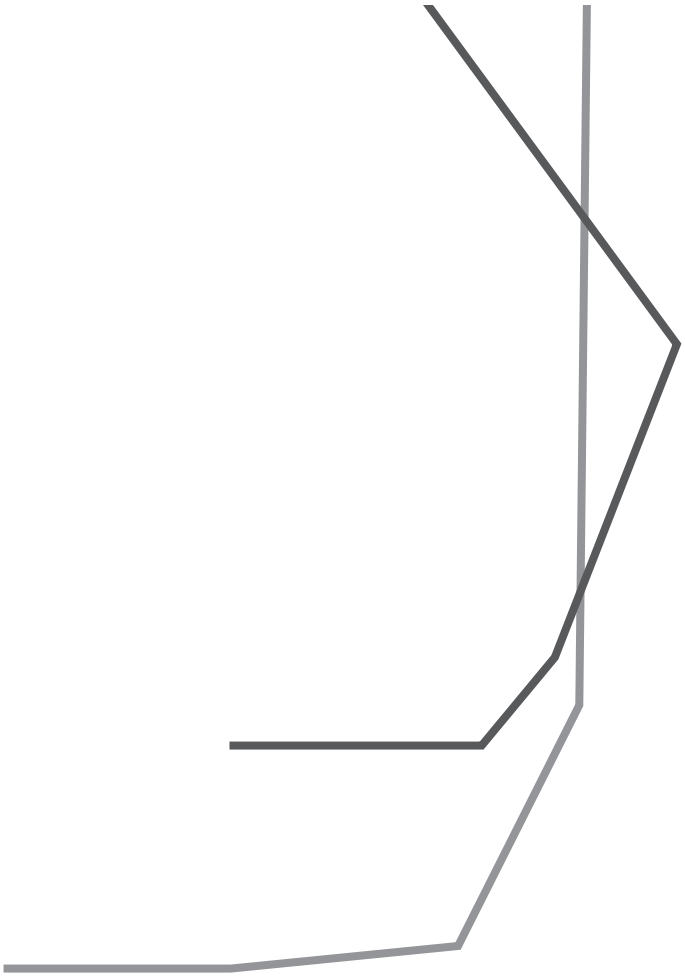

Chapter 4

Reply: Value of serial ST2 measurements in acute heart failure: miRNA regulation and genetic factors

L.C. van Vark, I. Kardys, E. Boersma, H.L. Hillege, K.M. Akkerhuis; TRIUMPH Investigators

J Am Coll Cardiol. 2018 Mar 27

We appreciate dr. Patanè's comments on our manuscript concerning the prognostic value of repeatedly measured ST2 in acute heart failure patients.[1] We agree that genetic factors influence the production of biomarkers such as ST2 [2] In a recent study, we investigated multiple microRNA's in the TRIUMPH cohort, which were selected because of their association with heart failure in previous studies, their enrichment in cardiomyocytes, or because they were muscle-specific. In our study of acute heart failure patients, baseline and repeatedly assessed microRNA-1306-5p was associated with adverse clinical outcome.[3] In our Bio-SHiFT study of 263 chronic heart failure patients repeatedly measured microRNA-22-3p contains important prognostic information, independent of clinical risk factors and (repeatedly measured) NT-proBNP, Troponin T and CRP.[4] MicroRNA-22 has not directly been associated with the ST2/IL-33 pathway, but plays a critical role in the regulation of cellular proliferation, differentiation, and stress-induced hypertrophy.[5]

Indeed, combining microRNA's that influence the ST2/IL-33 pathway, the variants of SNPs within IL1RL1 (the gene encoding ST2), and repeatedly measured ST2 may further clarify the role of ST2 in heart failure patients, as suggested by dr. Patanè. Information on genetic traits, in combination with temporal changes in biomarker levels may enable individualized prognostication and, ultimately, treatment response.

Disclosures

None.

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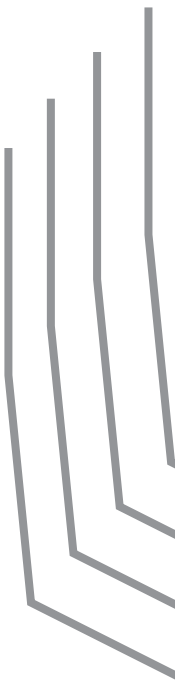

Chapter 6

Serially-measured circulating microRNAs and adverse clinical outcomes in patients with acute heart failure

N. van Boven, I. Kardys, L.C. van Vark, K.M. Akkerhuis, M.W.J. de Ronde, M.A.F. Khan, D. Merkus, Z. Liu, A.A. Voors, F.W. Asselbergs, E.J. van den Bos, E. Boersma, H.L. Hillege, D.J. Duncker, Y.M. Pinto, D. Postmus

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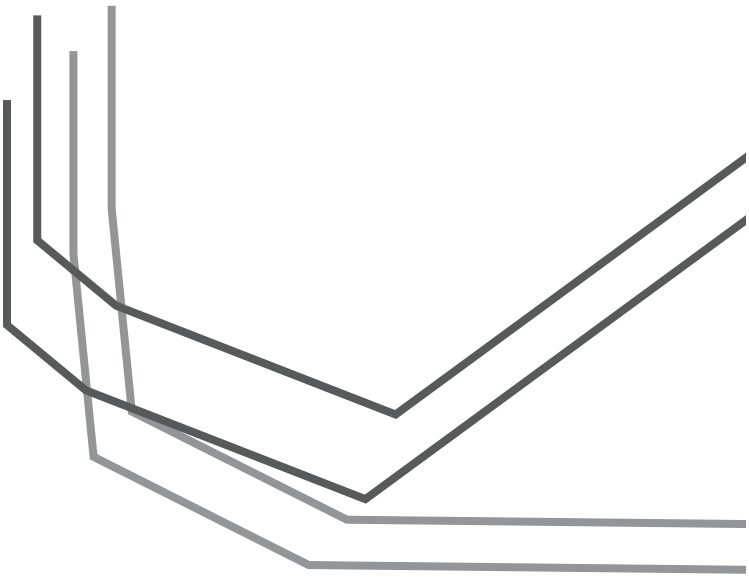

Abstract

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 upregulation 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) 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.

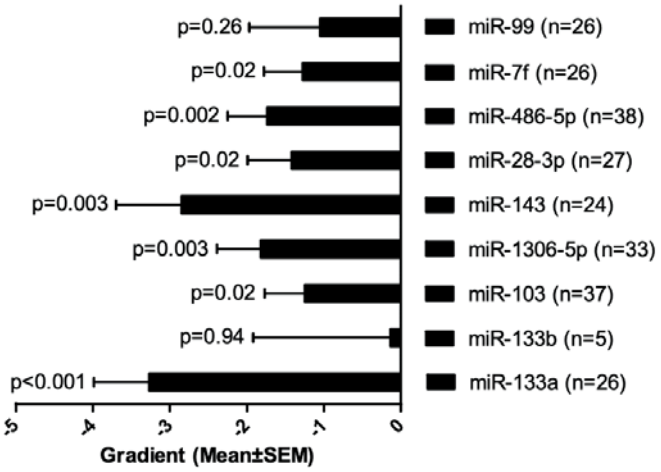

Figure 1. Trans-coronary gradients in plasma microRNAs
The number indicates the number of pigs (out of a total of 44 pigs) with both a detectable venous and arterial microRNA value. The gradient is calculated as arterial minus venous Ct value of the microRNA, and shown as Mean±SEM. A negative value indicates 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.

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

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 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.

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 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 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.

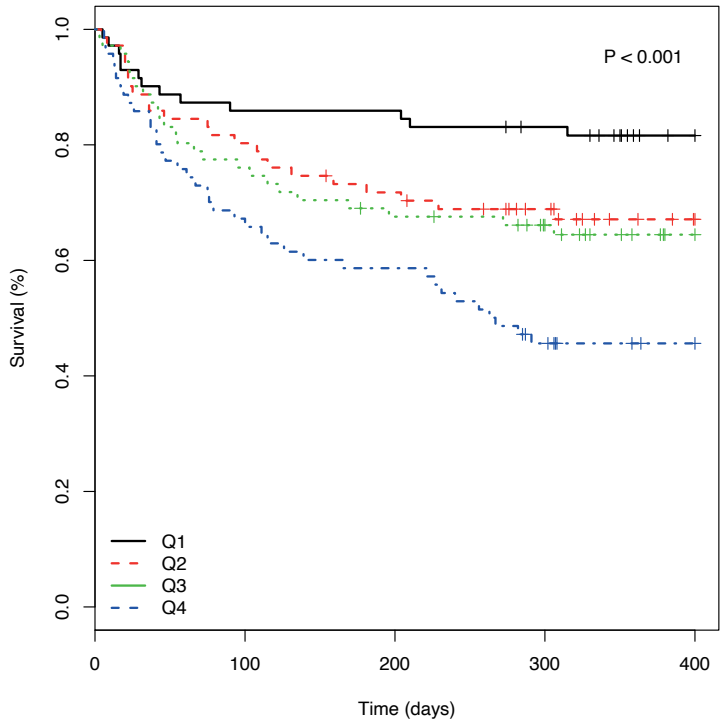

Figure 2. Kaplan-Meier survival curves for the primary endpoint of death or readmission for HF in the four quartiles of baseline miR-1306-5p levels
Q1 lowest quartile, Q4 highest quartile.

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 , CI = Confidence interval. 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

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.

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.

Table 4. Associations between repeated microRNA measurements and primary endpoint

miR	Hazard ratio (95% CI)		
	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 , CI = Confidence interval. Primary endpoint: composite of all-cause mortality and readmission for heart failure
Hazard ratio per per ln[arbitrary unit] of miR level

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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Supplement

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 108.
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)
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

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 CV (%)	Detroit CV (%)	mean CV (%)	Rank CV	Barcelona Final M-value	Detroit Final M-value	Mean Final M-value	Rank M-value	Rank CV	Rank M val	mean rank	final rank
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.

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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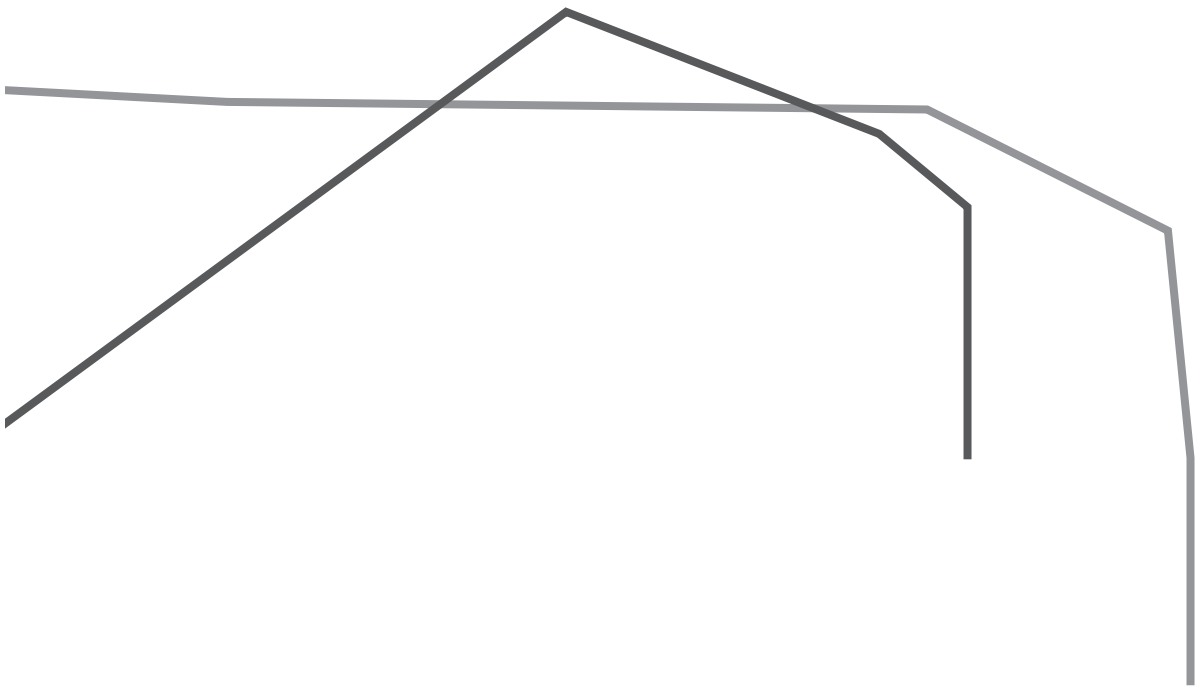

Chapter 7

Lipoprotein-associated phospholipase A2 activity and risk of heart failure: The Rotterdam study

L.C. van Vark, I. Kardys, G.S. Bleumink, A.M. Knetsch, J.W. Deckers, A. Hofman, B.H. Stricker, J.C. Witteman

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Abstract

Aims

Evidence is accumulating that inflammation plays a role in the pathophysiology of heart failure. Lipoprotein-associated phospholipase A2 (Lp-PLA2) has pro-inflammatory properties. We investigated whether Lp-PLA2 activity is associated with heart failure.

Methods and results

Lp-PLA2 activity was determined in a random sample of 1820 subjects from the Rotterdam Study, a population-based cohort study among persons aged 55 years and over. During a mean follow-up of 6.7 years, 94 heart failure cases occurred. We excluded participants with heart failure or coronary heart disease at baseline and we accounted for incident coronary heart disease during follow-up. We used Cox proportional hazard models to compute hazard ratios adjusted for age, sex, non-HDL cholesterol, HDL cholesterol, body mass index, systolic blood pressure, diastolic blood pressure, hypertension, diabetes mellitus, smoking, and C-reactive protein. The hazard ratio per unit increase of Lp-PLA2 activity was 1.03 [95% confidence interval (95% CI 1.01–1.05]; P for trend was 0.011. Hazard ratios for the second, third, and fourth quartiles were 1.06 (95% CI 0.55–2.04), 1.43 (95% CI 0.73–2.81), and 2.33 (95% CI 1.21–4.49), respectively, using the lowest quartile of Lp-PLA2 activity as the reference category.

Conclusion

This study suggests that Lp-PLA2 activity is independently associated with incident heart failure.

Introduction

During the last 15 years, an interest has developed for the potential role of inflammatory mediators in the pathophysiology of heart failure. Associations have been found between elevated inflammatory markers, such as interleukin-6,[1] tumour necrosis factor-α,[2] and C-reactive protein,[3] and congestive heart failure. It has been shown that inflammatory mediators may influence left ventricular remodelling, left ventricular function, and pulmonary oedema.[4,5] Furthermore, a correlation has been found between high blood levels of these inflammatory markers and worsening functional NYHA class, increased hospitalization rates, and poorer survival of heart failure patients.[6]

Recently, several studies have found an independent association between the inflammatory marker lipoprotein-associated phospholipase A2 (Lp-PLA2) and risk of coronary heart disease.[7,8,9,10,11] Lp-PLA2 is an enzyme that circulates in the blood bound to low-density lipoprotein (LDL) cholesterol. The enzyme has pro-inflammatory properties because of its capacity to hydrolyse oxidized phospholipids.[12] However, it is also suggested to have anti-inflammatory properties because of its ability to hydrolyse platelet-activating factor.[13,14] The relationship found between Lp-PLA2 and coronary heart disease suggests that the pro-inflammatory properties of Lp-PLA2 outweigh its anti-inflammatory properties.

To our knowledge, no studies have yet been conducted on Lp-PLA2 as a predictor of heart failure. Therefore, we investigated the association between Lp-PLA2 and risk of heart failure in the Rotterdam Study, a population-based cohort study among men and women aged 55 years and over.

Methods

Rotterdam Study

The Rotterdam Study is a population-based cohort study comprising 7983 men and women aged 55 years and over. Its overall aim is to assess the occurrence of and risk factors for chronic diseases in the elderly. A detailed description of the objectives and methods of the Rotterdam Study has been given elsewhere.[15] All residents of a Rotterdam suburb aged 55 and over were invited to participate in the study and 78% participated. Baseline measurements started in 1990 and were completed in 1993.

The Medical Ethics Committee of Erasmus Medical Center, Rotterdam, approved the

study. All participants gave written informed consent. This study complies with the Declaration of Helsinki.

Study population

Lp-PLA2 activity was determined in a random subcohort of 1820 subjects. Prevalent heart failure cases at baseline (n = 47) were excluded for the current analysis. In addition, 183 subjects were excluded because they had a history of myocardial infarction, a history of coronary artery bypass grafting (CABG), or a history of percutaneous transluminal coronary angioplasty (PTCA) at baseline, leaving 1590 subjects, who were used for the analysis.

Measurement of Lp-PLA2 activity

Plasma aliquots prepared from non-fasting blood samples were collected at baseline and stored at -80°C. Lp-PLA2 activity was measured with a high throughput radiometric activity assay, as described in detail previously.[11] Lp-PLA2 activity was expressed as nano moles of platelet-activating factor hydrolysed per minute per millilitre of plasma samples.

Prior to analysis of plasma samples from the Rotterdam Study, a pre-study validation was conducted to determine the reliability of the LpPLA2 activity assay. Six plasma samples were tested in triplicate, and the coefficient of variation (CV) for intra-assay precision ranged from 3.51–8.96%. To assess inter-assay precision, six plasma samples were tested on three occasions, and CV ranged from 8.48–15.08%. Three cycles of freeze–thaw of frozen plasma did not result in appreciable loss of activity. The assay was therefore considered suitable for the analysis of the Rotterdam Study samples, which were tested in duplicate. Samples were re-tested if the replicate CV was > 25%. The range of detection was 8–150 nmol/min per mL.

Measurement of covariates at baseline

At baseline, a trained interviewer visited all participants at home and collected information using a computerized questionnaire. The information obtained included current health status, medical history, drug use, and smoking behaviour. Additionally, established cardiovascular risk factors were measured at the research centre. Height and weight were measured, and the body mass index was calculated [weight (kg)/ height^2 (m^2)]. Blood pressure was measured at the right brachial artery with a random-zero sphygmomanometer, with the participant seated. We defined hypertension as a systolic blood pressure ≥ 160 mmHg or a diastolic blood pressure ≥ 100mmHg or the use of blood pressure-lowering medication with an indication for hypertension.

Non-fasting blood samples were drawn, and total cholesterol and high-density lipoprotein (HDL) cholesterol were measured within 2 weeks, as described previously.[16] Non-HDL cholesterol was computed by subtracting HDL cholesterol from total cholesterol. LDL cholesterol was determined in fasting blood samples in 120 randomly selected subjects by use of an enzymatic method (Roche). We calculated Pearson's correlation coefficient to compute the correlation of non-HDL cholesterol with LDL cholesterol, $r = 0.97$, $P < 0.001$. We defined diabetes mellitus as a random or post-load glucose level ≥ 11.1 mmol/L or the use of blood glucose-lowering medication.[17] Using a nephelometric method (Immage, Beckman Coulter), we measured C-reactive protein in blood samples kept frozen at -20°C.

A 12-lead resting electrocardiography (ECG) was recorded with an ACTA electrocardiograph (ESAOTE, Florence, Italy) at a sampling frequency of 500 Hz and stored digitally. All ECGs were processed by the modular ECG analysis system (MEANS) to obtain ECG measurements and interpretations.[18] Myocardial infarction found on ECG was based on a comprehensive set of criteria that partly derive from the Minnesota code. [19] A history of myocardial infarction was considered present in case of a self-report of myocardial infarction confirmed by ECG or additional clinical information, or the presence of an ECG characteristic of prior myocardial infarction.

In identifying incident myocardial infarctions (ICD-10 code I21), all available information, which included ECG, cardiac enzyme levels, and the clinical judgement of the treating specialist, was used. Revascularization procedures were identified by review of hospital discharge letters from the medical specialist.

Ascertainment of heart failure cases

Assessment of prevalent heart failure at the baseline examination in the Rotterdam Study has been described elsewhere in detail.[20, 21] Briefly, a validated score was used, similar to the definition of heart failure of the European Society of Cardiology.[22] This score was based on the presence of at least two symptoms suggestive of heart failure (shortness of breath, ankle swelling, and pulmonary crepitations) or use of medication for the indication of heart failure, in combination with objective evidence of cardiovascular disease.

Information on the presence of heart failure at baseline was obtained for all participants, using one of the following three methods: interview questions on indication of cardiovascular medication and breathlessness, linkage of the Rotterdam Study database to a database containing hospital discharge diagnoses from all hospitals in the

Rotterdam area as of 1 January 1991, and screening of all medical records in retrospect for the occurrence of heart failure in the majority of participants of the Rotterdam Study.

For the present study, follow-up started at the baseline examination, from 1990 till 1993, and was complete until 1 January 2000. Follow-up has been described in detail previously.[21] Briefly, cases of incident heart failure were obtained by continuously monitoring participants of the Rotterdam Study for the occurrence of heart failure during follow-up through automated linkage with files from general practitioners. Each participant's medical record was fully screened for incident heart failure. All available data on these events, such as hospital discharge letters and notes from general practitioners, were copied from the medical records. Apart from this systematic follow-up procedure, we used verified hospital discharge diagnoses for case finding, gathered from all hospitals in the Rotterdam area, as described earlier. The diagnosis of heart failure was classified as definite, probable, possible, or unlikely. [21] Two research physicians independently classified all information on potential heart failure events. If there was disagreement, a consensus was reached in a separate session. Finally, a cardiologist verified all probable and possible cases, and all cases in which the two physicians could not reach consensus. If the cardiologist disagreed with the research physicians, the cardiologist's judgement was considered decisive. The research physicians and the cardiologist based their decisions on the same data. Only definite and probable cases were included in the analyses.

Statistical analysis

To compare the baseline characteristics of the random subcohort to the remainder of the Rotterdam Study, we used a χ^2 test for dichotomous variables, a t-test for continuous variables, and a Mann– Whitney test for C-reactive protein, because its distribution was skewed. We used ANCOVA to display age- and sex-adjusted baseline characteristics of the participants in different Lp-PLA2 activity quartiles. We log-transformed C-reactive protein because of its skewed distribution and we computed the geometric mean. We computed the standard deviation and interquartile range from the standard error. To compute P-value for trend for the baseline characteristics, we used logistic regression for dichotomous variables and linear regression for continuous variables. In both cases, continuous plasma values of Lp-PLA2 activity were used as the independent variable.

We used Cox proportional hazards models to evaluate the association of Lp-PLA2 activity with risk of heart failure. Subjects were censored at the time of occurrence of heart failure, death, or at the end of the study period. Furthermore, we censored subjects at the time of occurrence of myocardial infarction, PTCA, or CABG if these took place before

the occurrence of heart failure, to account for coronary heart disease. The proportional hazards assumption was tested by drawing log minus log plots of the survival function, which confirmed that the assumption was met. In model 1, we adjusted for age and sex. Lp-PLA2 is tightly associated with lipoproteins; in humans, it is predominantly located on LDL and, to a smaller extent, on HDL. Because these factors were most likely to be confounders, in model 2, we additionally adjusted for non-HDL cholesterol and HDL cholesterol. In model 3, we additionally adjusted for body mass index, systolic blood pressure, diastolic blood pressure, hypertension, smoking status, diabetes mellitus, and C-reactive protein. First, we entered the continuous plasma values of Lp-PLA2 activity into the models to obtain the hazard ratio for heart failure per unit increase in Lp-PLA2 activity. By this means, we also obtained the P-value for trend. Second, to allow for the demonstration of a possibly non-linear association, we made quartiles of Lp-PLA2 activity with cutpoints 35.9, 42.9, and 50.8 nmol/min per mL plasma and used the lowest quartile as the reference category.

To compare survival time until the occurrence of heart failure in the quartiles of Lp-PLA2 activity, C-reactive protein, and non-HDL cholesterol, we made event-free survival curves adjusted for age and sex.

We conducted a subgroup analysis to compare the association between Lp-PLA2 activity and heart failure in subjects with a non-HDL cholesterol level below and above the median (cutpoint 5.20 mmol/L). Lp-PLA2 was dichotomized in this analysis, using the median as a cutoff point (42.9 nmol/min per mL plasma). We adjusted for age, sex, HDL cholesterol, body mass index, systolic blood pressure, diastolic blood pressure, hypertension, smoking status, diabetes mellitus, and C-reactive protein. We did a similar subgroup analysis in the strata of C-reactive protein (cutpoint 1.79 mg/L), adjusting for non-HDL cholesterol instead of C-reactive protein in the Cox proportional hazard model.

To test for interaction between Lp-PLA2 activity and non-HDL cholesterol and C-reactive protein, we entered interaction terms into the model, using continuous values of Lp-PLA2 activity instead of quartiles of Lp-PLA2 activity and using non-HDL cholesterol and C-reactive protein as continuous variables. In this analysis, we adjusted for age and sex. Values for covariates were missing in < 3%, except for C-reactive protein (6% missing values). We used single imputation based on expec- tation maximization to handle missing values.

Results

The mean follow-up time until censoring was 6.7 years (SD 2.3 years). During follow-up, 113 incident heart failure cases occurred. Of these cases, 19 were preceded by coronary heart disease. Therefore, 94 incident cases of heart failure were left for analysis.

Table 1 shows the baseline characteristics of the total random cohort and the remainder of the Rotterdam Study. The characteristics of the random cohort were similar to the remainder of the Rotterdam Study, except for age, systolic blood pressure, and hypertension. Subjects in the random cohort were slightly younger (69.1 vs. 71.1 years), had a lower systolic blood pressure (138.2 vs. 139.9 mmHg.), and had a lower prevalence of hypertension (33.1 vs. 37.1%). Table 2 shows the baseline characteristics of participants in different quartiles of Lp-PLA2 activity adjusted for age and sex (when appropriate) and the P-value for trend. In all linear regression models we used, the residuals were normally distributed with a constant variance. Quartiles with a higher Lp-PLA2 activity contained a higher percentage of men and hypertensive participants. They had a significantly higher body mass index, systolic blood pressure, non-HDL cholesterol, and C-reactive protein. HDL cholesterol was lower in participants within higher quartiles of Lp-PLA2 activity.

Lp-PLA2 activity was associated with risk of heart failure (Table 3). After adjustment for age and sex, the hazard ratio for heart failure per unit increase in Lp-PLA2 activity was 1.02 [95% confidence interval (CI) 1.00–1.03], P = 0.026. After additional adjustment for non-HDL cholesterol and HDL cholesterol, this was 1.02 (95% CI 1.00–1.04), P = 0.024, and after additional adjustment for known cardiovascular risk factors, 1.03 (95% CI 1.01–1.05), P = 0.011. Participants in the second, third, and fourth quartiles of Lp-PLA2 activity had a hazard ratio of 0.99 (95% CI 0.52–1.86), 1.27 (95% CI 0.68–2.40), and 1.93 (95% CI 1.09–3.42), respectively, for heart failure, using the first quartile of Lp-PLA2 activity as the reference category and adjusting for age and sex. Using model 2, this was 1.01 (95% CI 0.53– 0.92), 1.34 (95% CI 0.69– 2.60), and 2.16 (95% CI 1.13–4.11), respectively. Using model 3, this further increased to 1.06 (95% CI 0.55–2.04), 1.43 (95% CI 0.73–2.81), and 2.33 (95% CI 1.21–4.49), respectively.

Table 1. Baseline characteristics.

Variable	Random Cohort (n = 1820)	Remainder Rotterdam Study (n = 6163)	p-value
Age, y	69.1 ± 9.1	71.1 ± 9.9	<0.01
Men, %	38.3	39.1	0.55
Body mass index, kg/m^2	26.2 ± 3.7	26.3 ± 3.8	0.29
Systolic blood pressure, mm Hg	138.2 ± 22.3	139.9 ± 22.4	<0.01
Diastolic blood pressure, mm Hypertension, %	73.3 ± 11.2	73.8 ± 11.8	0.12
Non-HDL cholesterol, mmol/L	33.1	37.1	<0.01
HDL-cholesterol, mmol/L	5.30 ± 1.24	5.24 ± 1.23	0.1
Diabetes mellitus, %	1.35 ± 0.38	1.34 ± 0.37	0.38
Smokers, %	9.8	10.7	0.31
Current			
Former	23.0	22.5	0.68
Never	41.7	40.4	0.34
C-reactive protein, mg/L	35.3	37.0	0.18
	1.78 (0.90-3.59)*	1.93 (0.92-3.71) *	0.07

Continuous variables are expressed as mean ± SD. Categorical variables are expressed as percentage.
* Median and interquartile range because of skewed distribution.

The event-free survival curve according to quartiles of Lp-PLA2 activity shows that the survival time until the occurrence of heart failure was higher in the lowest quartile than in the highest quartile (Figure 1). The curve also illustrates that the difference in risk between quartiles 1 and 4 is rather consistent over time. Figures 2 and 3 show the event- free survival curve of C-reactive protein and non-HDL cholesterol, respectively. Although C-reactive protein was significantly related to the event-free survival time, no clear association was found for non-HDL cholesterol.

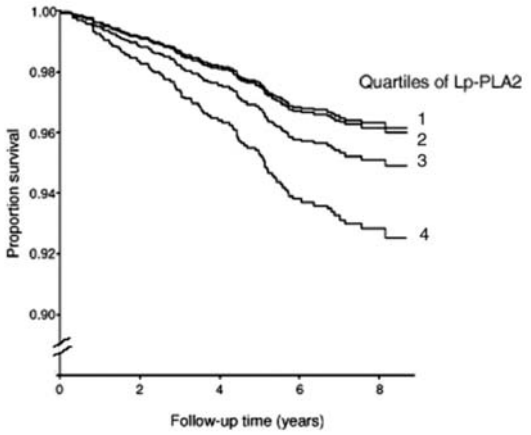

Figure 1. Survival time until the occurrence of heart failure according to quartiles of Lp-PLA2, adjusted for age and sex.

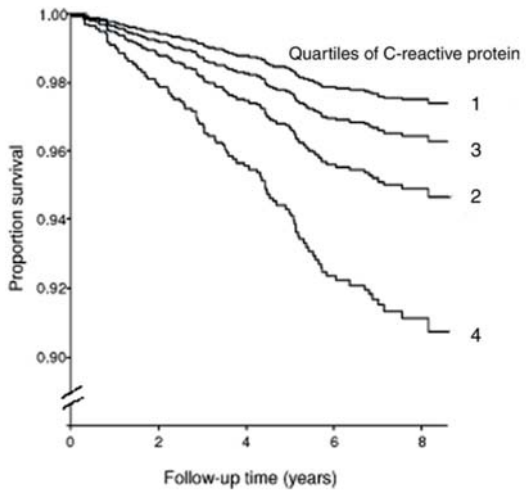

Figure 2. Survival time until the occurrence of heart failure according to quartiles of C-reactive protein, adjusted for age and sex.

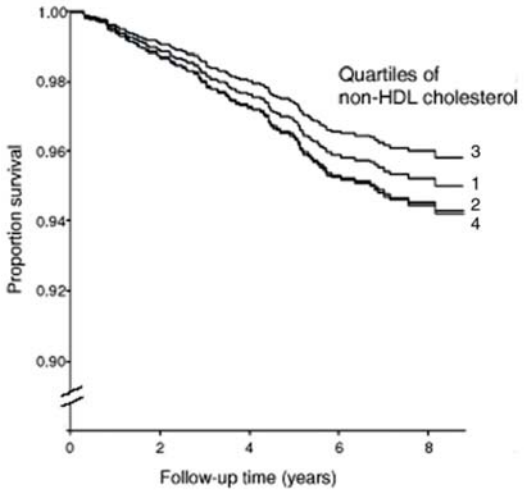

Figure 3. Survival time until the occurrence of heart failure according to quartiles of non-HDL cholesterol, adjusted for age and sex.

Figure 4 shows the results of our subgroup analyses. The hazard ratios for heart failure associated with Lp-PLA2 activity for the subgroups below and above the median of non-HDL cholesterol level were 1.89 (95% CI 1.05–3.39) and 1.77 (95% CI 0.83–3.79), respectively. The hazard ratio was somewhat larger in subjects with a non-HDL

cholesterol below the median, but no significant interaction was found between Lp-PLA2 activity and non-HDL cholesterol (P-value for interaction = 0.817) in relation to risk of heart failure. The hazard ratio for the subjects with a C-reactive protein below the median was 3.83 (95% CI 1.64–8.93), which was higher than the hazard ratio for the subjects with C-reactive protein above the median, namely 1.26 (95% CI 0.71–2.23). However, the interaction term for Lp-PLA2 activity and C-reactive protein was not significant (P-value for interaction = 0.364).

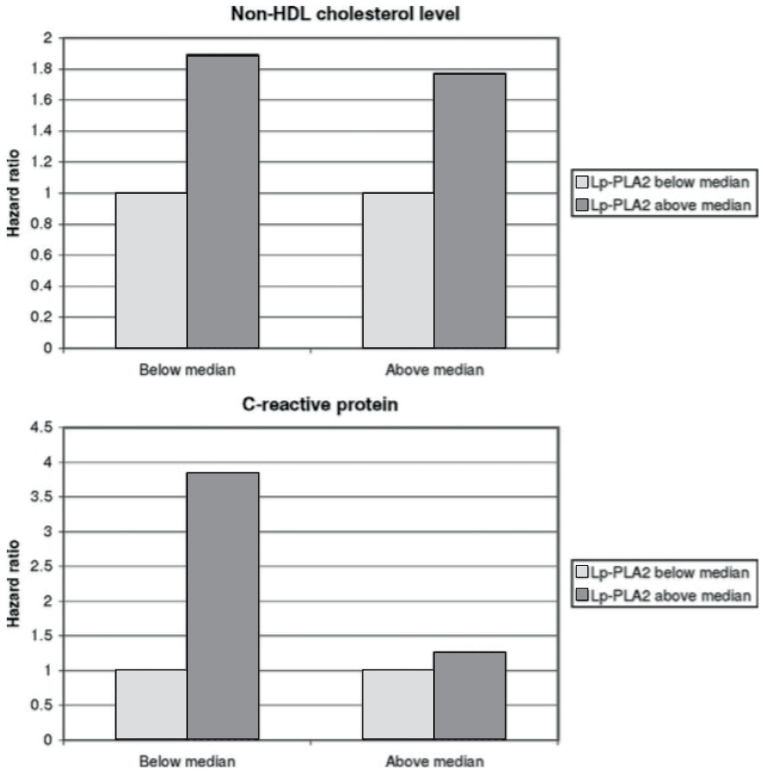

Figure 4. Hazard ratios for heart failure associated with Lp-PLA2 activity in strata of non-HDL cholesterol level and C-reactive protein.

Table 2. Baseline characteristics according to quartiles of Lp-PLA2 activity.

Variables	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-value for trend
Age, years	68.6 ± 9.1	69.7 ± 9.0	68.8 ± 9.0	69.5 ± 9.1	0.29
Men, %	27.1	35.2	42	48.9	<0.01
Body mass index, kg/m2	25.8 ± 3.6	26.1 ± 3.6	26.5 ± 3.6	26.5 ± 3.6	<0.01
Systolic blood pressure, mmHg	135.9 ± 21.5	138.4 ± 21.4	137.7 ± 21.4	140.8 ± 21.5	<0.01
Diastolic blood pressure, mmHg	72.8 ± 11.2	73.8 ± 11.2	73.4 ± 11.2	73.3 ± 11.2	0.84
Hypertension, %	29	30.7	33.3	39.5	<0.01
Non-HDL cholesterol, mmol/L	4.49 ± 1.09	5.13 ± 1.08	5.53 ± 1.06	6.06 ± 1.08	<0.01
HDL cholesterol, mmol/L	1.50 ± 0.36	1.38 ± 0.36	1.28 ± 0.36	1.25 ± 0.36	<0.01
C-reactive protein, mg/L	1.67 (1.62–1.73)*	1.69 (1.64–1.75)*	1.83 (1.77–1.89)*	1.97 (1.91–2.04)*	<0.01
Diabetes, %	8.8	9.6	11	10	0.18
Smokers, %					
Current	24.5	20.6	22.5	24.3	0.76
Former	42.7	44	42.7	37.5	0.10
Never	32.8	35.4	34.8	38.2	0.05

Continuous variables are expressed as mean ± standard deviation. Categorical variables are expressed as percentage. All (geometric) means and percentages are adjusted for age and sex, except for age (only adjusted for sex) and sex (only adjusted for age). *Geometric mean and interquartile range because of skewed distribution.

Table 3. Hazard ratios for heart failure according to quartiles of Lp-PLA2 activity

Lp-PLA2 (nmol/min per mL)	Cases/ subjects	Hazard ratio (95% CI)		
		Model 1	Model 2	Model 3
Unit increase	94/1590	1.02 (1.00 – 1.03)	1.02 (1.00 – 1.04)	1.03 (1.01 – 1.05)
P-value for trend		0.026	0.024	0.011
Quartile 1	18/397	1 (reference)	1 (reference)	1 (reference)
Quartile 2	20/398	0.99 (0.52 – 1.86)	1.01 (0.53 – 1.92)	1.06 (0.55 – 2.04)
Quartile 3	21/398	1.27 (0.68 – 2.40)	1.34 (0.69 – 2.60)	1.43 (0.73 – 2.81)
Quartile 4	35/397	1.93 (1.09 – 3.42)	2.16 (1.13 – 4.11)	2.33 (1.21 – 4.49)

Model 1 adjusted for age and sex; model 2 adjusted for age, sex, non-HDL cholesterol, and HDL cholesterol; model 3 adjusted for age, sex, non-HDL cholesterol, HDL cholesterol, body mass index, systolic blood pressure, diastolic blood pressure, hypertension, diabetes mellitus, smoking status, and C-reactive protein.

Discussion

In the present population-based cohort study, Lp-PLA2 activity was an independent predictor of heart failure. The association persisted after we adjusted for known cardiovascular risk factors and C-reactive protein. A significant trend was seen, and

subjects in the highest quartile had no less than a doubled risk of developing heart failure compared with subjects in the lowest quartile, even though we excluded subjects with prevalent coronary heart disease at baseline and censored subjects with incident coronary heart disease during follow-up. This suggests that the association found between Lp-PLA2 activity and heart failure is independent of coronary heart disease.

To our knowledge, this is the first study performed on the association between Lp-PLA2 and risk of heart failure. The present study is a population-based prospective cohort study, which guards our study from selection and recall bias. Strengths of our study include the ability to account for possible confounding by incorporating established cardiovascular risk factors into the statistical models. Finally, we were able to account for prevalent and incident coronary heart disease in our analysis.

The pathophysiology of heart failure is complex. Heart failure was once considered to be merely a cardiocirculatory impairment, but now it is known that the neuroendocrine system is involved. Evidence is accumulating that inflammation also plays a direct role in the pathophysiology of heart failure. In former studies, several inflammatory markers, such as interleukin-6,[1] tumour necrosis factor-α,[2] and C-reactive protein,[3] have been associated with incidence of heart failure. Inflammatory mediators have been found to affect left ventricular remodelling, left ventricular dysfunction, pulmonary oedema, fetal gene expression, and cardiomyopathy.[4,5] Finally, a correlation has been found between high blood levels of inflammatory markers and poorer prognosis in heart failure patients.[6]

In the Atherosclerosis Risk in Communities study, an association between Lp-PLA2 and incident coronary heart disease was present after adjustment for age, sex, and race. After further adjustments for cardiovascular risk factors, the association was only present in subjects with a low LDL cholesterol.[9] Our subgroup analysis showed that the association found between Lp-PLA2 activity and heart failure is present in subjects with a non-HDL cholesterol level below the median as well as in subjects with a non-HDL cholesterol level above the median, although in the latter group, the association lacked significance. The association between Lp-PLA2 and heart failure was much stronger in subjects with a C-reactive protein level below the median than in subjects with a C-reactive protein level above the median. We have no explanation for this difference in risk estimates. The interaction between Lp-PLA2 and C-reactive protein was not significant, so the difference may be due to chance. Several studies have investigated the association between LDL cholesterol and heart failure [23-25]

Although the Framingham Study found a positive relation,[26] subsequent studies were not able to confirm this. In our study, we also failed to find a clear relation between non-HDL cholesterol and risk of heart failure. Lp-PLA2 is an enzyme bound to LDL cholesterol and therefore Lp-PLA2 activity is highly cor- related with LDL cholesterol levels. In the present study, we found that the association of Lp-PLA2 with heart failure was independent of non-HDL cholesterol. We used non-HDL cholesterol for adjustment, since no measurements of LDL cholesterol were available. Because of the high correlation between LDL cholesterol and non-HDL cholesterol in a random sample of our cohort ($r = 0.97$, $P < 0.001$), we believe that residual confounding by LDL cholesterol cannot explain our results.[11]

In conclusion, our findings suggest that Lp-PLA2 activity is independently associated with risk of heart failure. Our study provides further evidence that inflammation is involved in the aetiology of heart failure.

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Conflict of interest

None declared.

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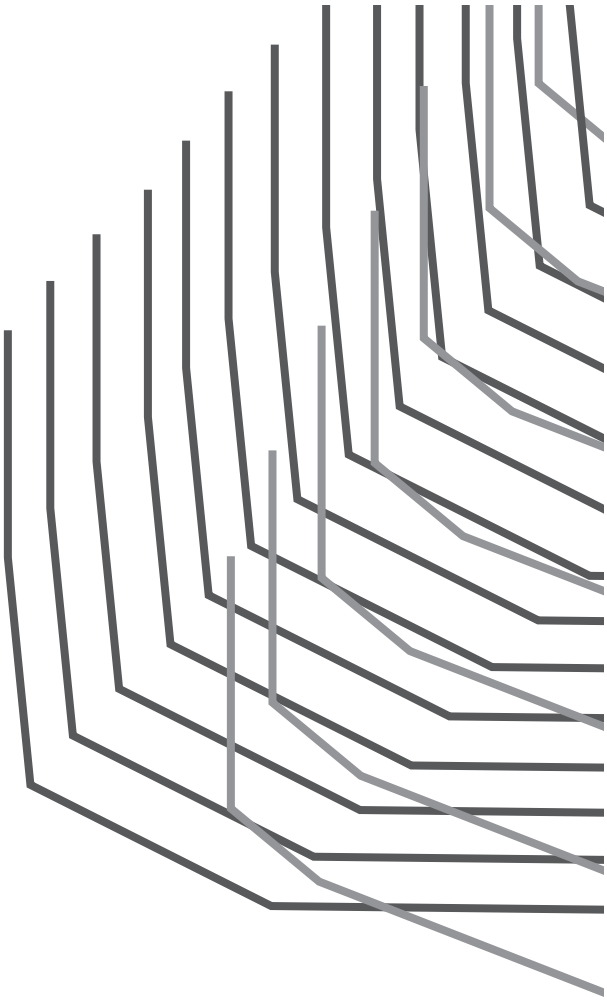

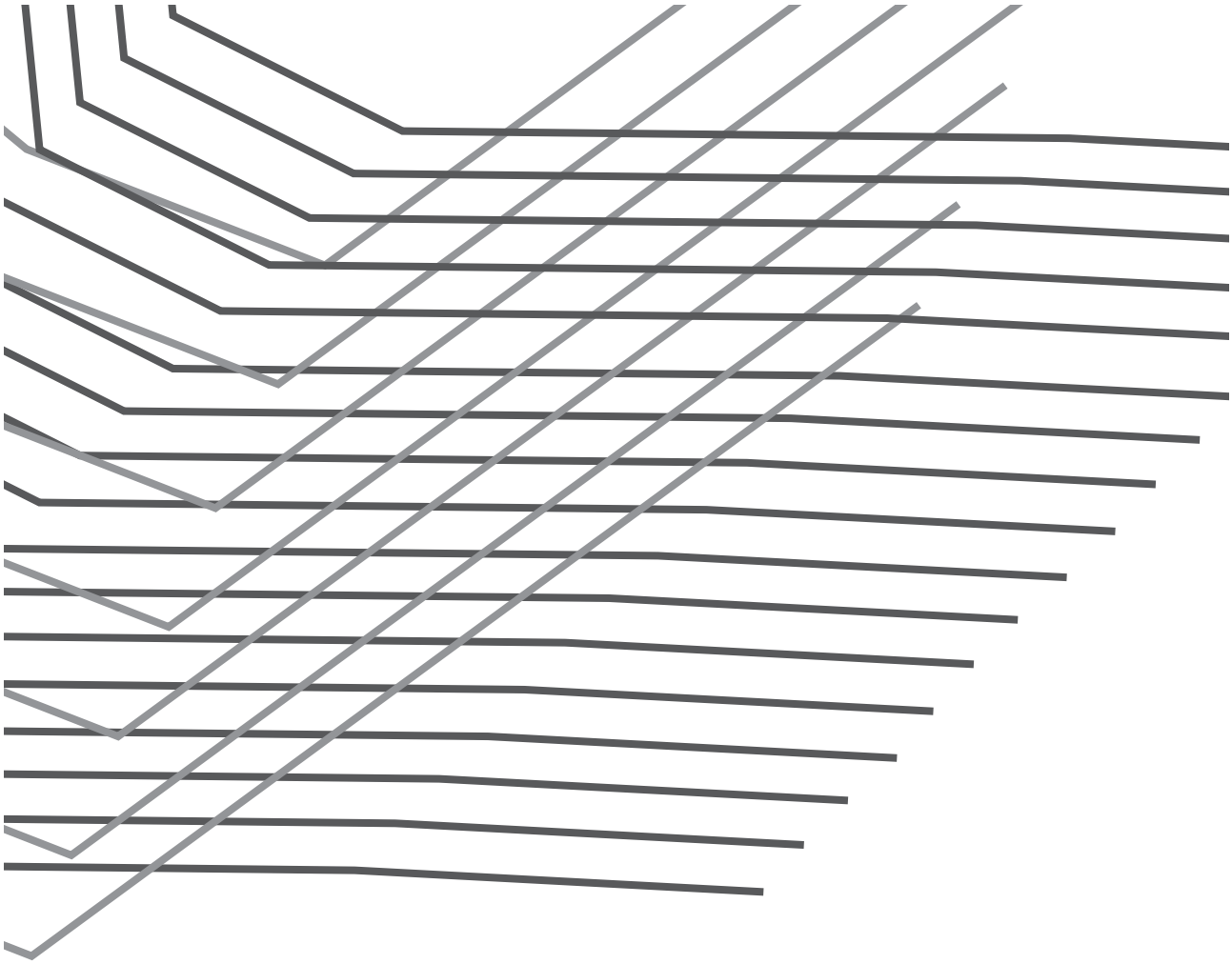

Chapter 8

Biomarkers of heart failure with normal ejection fraction: a systematic review

J.M. Cheng, K.M. Akkerhuis, L.C. Battes, L.C. van Vark, H.L. Hillege, W.J. Paulus, E. Boersma, I. Kardys

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Abstract

Aims

Heart failure with normal ejection fraction (HFNEF) is a major and growing public health problem, currently representing half of the heart failure burden. Although many studies have investigated the diagnostic and prognostic value of new biomarkers in heart failure, limited data are available on biomarkers other than natriuretic peptides in HFNEF. We performed a systematic review of epidemiologic studies on the associations of biomarkers with the occurrence of HFNEF and with the prognosis of HFNEF patients.

Methods and results

Biomarkers examined most extensively in HFNEF include biomarkers of myocyte stress, inflammation and extra-cellular matrix remodeling. Some biomarkers have been shown to be increased to a different extent in HFNEF compared to heart failure with reduced ejection fraction (HFREF). Several biomarkers, including biomarkers of myocyte stress, inflammation, extracellular matrix remodeling, growth differentiation factor 15 (GDF-15), cystatin C, resistin and galectin-3 were associated with development of HFNEF and with clinical outcomes of HFNEF patients in terms of morbidity and mortality.

Conclusion

Several biomarkers, including biomarkers of myocyte stress, inflammation, extracellular matrix remodeling, growth differentiation factor 15 (GDF-15), cystatin C, resistin and galectin-3 appeared to be promising diagnostic and prognostic tools in patients with HFNEF. Investigation of the incremental diagnostic and prognostic value of these biomarkers, or a combination thereof, over established clinical covariates and imaging techniques in large, prospective studies is warranted.

Introduction

Heart failure (HF) is a major and growing public health problem that is associated with substantial morbidity and mortality.[1,2] Classically, HF has been considered to be associated with impaired cardiac contractility and cardiac dilatation. In the past decade, however, it has become evident that a considerable portion of patients presenting with clinical HF have a normal left ventricular ejection fraction (LVEF). Some studies report a prevalence as high as 50%.[3] This entity is often termed HF with normal ejection fraction (HFNEF), sometimes also referred to as HF with preserved ejection fraction.[1,2] Despite improvements in understanding the underlying disease mechanisms, the exact mechanism and the classification of HFNEF are still debated.[3] In the single syndrome hypothesis, HFNEF and HF with reduced LVEF (HFREF) are viewed as two ends of one HF spectrum, the major difference being the degree of left ventricular dilatation and shape change or left ventricular remodelling.[3] Although HFNEF is typically characterized by the presence of diastolic dysfunction, HFREF is found to be associated with reduced myocardial tissue doppler velocities as well, which supports the single HF syndrome hypothesis. On the other hand, the theory has been proposed that clinical HF presents and evolves not as a single syndrome but as two syndromes, one with depressed LVEF and the other with normal LVEF and specific mechanisms responsible for diastolic LV dysfunction, this theory being supported by structural, functional, and molecular biological arguments.[3] Regardless of which hypothesis will eventually turn out to be appropriate, the prognosis after hospitalization for HFNEF appears to be as ominous as that of HFREF with a mortality rate of approximately 65% at 5 years.[4]

Although echocardiography is the most useful noninvasive diagnostic method for evaluating systolic and diastolic dysfunction, current state-of-the-art echocardiography has limited value for prognostication in HF.[1,2,5] HF results from a complex interplay between genetic, neurohormonal, inflammatory and biochemical changes acting on cardiac myocytes and the cardiac interstitium. Thus, the sequence of events that lead to overt changes in the ventricle begins at cellular level, and assessing these phenomena could be of greater value to improve prognostication. Biomarkers, in this context meaning proteins measured in blood, may play an important part in this respect. Biomarkers may provide important information on the pathogenesis of HF, but may also be a valuable clinical tool in the identification of patients at risk for HF, in the diagnosis of HF, in risk stratification and in monitoring therapy. Furthermore, reliable non-invasive measures of pre-symptomatic worsening of (diastolic) ventricular function, including biomarkers, could aid in prevention of ensuing decompensation with its adverse sequels.

Although many studies have investigated the diagnostic and prognostic value of new biomarkers in HF, the majority of these studies included HFREF patients only.[5] Limited data are available on biomarkers in HFNEF. This review provides a thorough, yet concise, overview of clinical and population based studies on the associations of biomarkers with occurrence of HFNEF and with prognosis of HFNEF patients, and of the pathophysiology underlying these associations. We summarize research on established biomarkers, such as natriuretic peptides, but we mainly focus on biomarkers of myocyte stress, inflammation and extra-cellular matrix remodeling since the body of evidence on these markers is less elaborate.

Methods

Using Medline (Pubmed U.S. National Library of Medicine), we performed a literature search from inception to February 2012 using the following search terms: "Heart Failure" (MeSH term) and "normal ejection fraction" or "HFNEF" or "preserved ejection fraction" or "HFPEF" or "diastolic heart failure" or "DHF" or "diastolic dysfunction" in combination with "Biological Markers" (MeSH term) or "cytokine" or "CRP" or "TNF" or "MMP" or "TIMP" or "collagen". We limited our search to studies on human adults. Articles were included if they fulfilled the following criteria: a study population that includes patients with HFNEF or that has registered incident HFNEF; measurement of biomarkers in blood samples (other than natriuretic peptides); and reference to outcome in terms of morbidity and mortality. In addition, references of included studies were checked to ensure that no potentially eligible studies were missed.

HFNEF was defined as a reported clinical diagnosis of HF as well as LVEF higher than a cutoff value of choice in the specific study, which could range from 40% to 55%. Studies that have reported on associations with diastolic dysfunction in general, but not on associations with HFNEF, were excluded.

Results

The systematic literature search yielded 198 potential eligible studies. After exclusion of the studies that did not fulfill our criteria, 26 original studies were included in this review. The study populations and baseline characteristics of these studies are shown in Table 1. Additionally, three literature reviews were found on the utility of natriuretic peptides in HFNEF.[6-8]

BIOMARKERS OF MYOCYTE STRESS

Natriuretic peptides

Among biomarkers of myocyte stress, brain natriuretic peptides (BNP) have been investigated most extensively.[5] Nevertheless, our understanding on the biochemistry of natriuretic peptides currently may not be fully complete, as exemplified by the occurrence of the "natriuretic peptide paradox". Pro-brain natriuretic peptide (proBNP) is synthesized by the heart in reaction to cardiac wall distension and stretching, and neurohormonal activation.[9] During secretion from the cardiomyocytes, the biologically inactive amino-terminal fragment (NT-proBNP) is split from proBNP. An increased active BNP concentration in the plasma leads to natriuresis, vasodilatation, inhibition of the renin-angiotensin system, adrenergic activity and improved myocardial relaxation. Herewith, BNP is expected to have an important regulatory role in response to acute increases in ventricular volume and overload. In HF patients, although levels of serum immunoreactive natriuretic peptides are already elevated, administration of BNP has additional beneficial effects.[10] This "natriuretic peptide paradox" may be explained by the fact that in HF patients, the fraction of active BNP in the blood is relatively small. This signifies that HF is characterized by altered natriuretic peptide processing with secretion of less biologically active forms, while proBNP is the major immunoreactive form that is measured by laboratory assays.[10]

The associations of the natriuretic peptides with HFNEF have already been evaluated extensively in recently published reviews.[6-8] As such, we will not evaluate these associations in depth in the current paper. In brief, the majority of data show that BNP and NT-proBNP levels are increased in both HFREF and HFNEF compared to control subjects. Higher plasma NT-proBNP levels are shown to be associated with greater severity of diastolic dysfunction in patients with HFNEF.[11] However, co-morbidities are major drivers of higher NT-proBNP levels in HFNEF as well.[12] Several studies have also shown that plasma levels of natriuretic peptides are strong predictors of mortality and hospitalizations in both patients with HFREF and patients with HFNEF.[13] For a given BNP level, the prognosis in patients with HFNEF is as poor as in those with HFREF.[14]

Table 1. Baseline characteristics.

	Year	Population (n)	EF cutoff for HFNEF	Sample size, n				Age, years ± SD			
				HFNEF	*HFREF*	*LVH, no HF*	*No HF or LVH*	*HFNEF*	*HFREF*	*LVH, no HF*	*No HF or LVH*
Yu et al. (15)	2001	hospitalized HF (77), healthy controls (17)	50%	31	46		17	66.5 ± 8.4	65.7 ± 12.2		
Amosova et al. (23)	2004	hospitalized HFNEF (26), healthy controls (10)	NR								
Wisniacki et al. (19)	2005	outpatient HF aged 70-90 (52), healthy (26)	50%	25	27		26	80.4 ± 4.5	79.8 ± 5.2		76,1 ± 3.5
Ahmed et al. (35)	2006	outpatient LVH patients (49), healthy controls (53)	50%	26		23	53				59 ± 7
Martos et al. (36)	2007	outpatient HT patients (86)	45%	32			54	72 ± 11			67 ± 9
Varol et al. (49)	2007	outpatient HCM patients with HF (32), healthy controls (30)	NR	32			30	51.3 ± 18.4			49.6 ± 16.1
Frantz et al. (37)	2008	hospitalized HF (249), healthy controls (74)	45%	102	147		74				
Michowitz et al. (17)	2008	outpatient HF (294), healthy controls (7701)	45%	77	217		7701	71 ± 11.2	72.4 ± 10.8		
Dunlay et al. (29)	2008	community-based HF patients (486)	50%	486				76.7 ± 13.0			
Moran et al. (44)	2008	community-based aged ≥65 (4453)	50%				4453				
Niethammer et al. (24)	2008	hospitalized HFNEF (17), hospitalized HFPEF (17), healthy controls (20)	50%	17	17		20	72 ± 9	70 ± 8		56 ± 5
Barasch et al. (40)	2009	community-based aged ≥65 (880)	55%	179	131		570	76 ± 5	77 ± 6		77 ± 6
Butler et al. (46)	2009	community-based aged 70-79 (2902)	40%				2902				73.6 ± 2.9
Naito et al. (38)	2009	hospitalized HF (110), hospitalized non-HF patients (10)	45%	42	68		10	74 ± 13	71 ± 16		70 ± 13
Okuyan et al. (18)	2010	hospitalized HFNEF (68), healthy controls (40)	50%	68			40	65.5 ± 9.6			65.2 ± 9.7
Stahrenberg et al. (42)	2010	community-based HF patients (228), healthy elderly controls (188)	50%	142	86		188	73 [66-78]	71 [66-75]		56 [52-63]
Gonzales et al. (39)	2010	outpatient hypertensive patients with HFNEF (156), healthy controls (20)	50%	156			20	75 ± 9			
Kalogeropoulos et al. (28)	2010	community-based aged 70-79 (2610)	45%				2610				73.6 ± 2.9
De Boer et al. (13)	2011	Hospitalized HF (592)	40%	114	368			74 ± 10	69 ± 12		
Matsubara et al. (16)	2011	hospitalized HF (181), hospitalized non-HF patients (171)	50%	82	70		171	71.2 ± 10.2	65.5 ± 13.6		66.5 ± 11.2
Carrasco-Sanchez et al. (45)	2011	hospitalized HFNEF (218)	45%	218				75.6 ± 8.7			
Wu et al. (25)	2011	hospitalized HFNEF (110), hospitalized non-HF patients (55)	NR	110			55	72.22 ± 9.86			72.16 ± 9.62
Zile et al. (26)	2011	outpatient LVH patients (205), healthy controls (241)	50%	61		144	241	66 ± 8		60 ± 12	58 ± 16
Collier et al. (27)	2011	outpatient HT patients (275)	50%	181			94	73 ± 12			66 ± 10
Krum et al. (41)	2011	outpatient HFNEF aged ≥60 (313)	45%	313				72 ± 7			
Santhanakrishnan et al. (43)	2012	In- and outpatient compensated HF patients (101), healthy controls (50)	50%	50	51		50	69 ± 12	59 ± 11		63 ± 8

Data are presented as percentage, mean ± standard deviation or as median [interquartile range].
DM = diabetes mellitus; EF = ejection fraction; HF = heart failure; HFNEF = heart failure with normal ejection fraction; HFREF = heart failure with reduced ejection fraction; HT = hypertension; LVF = left ventricular hypertrophy; NR = not reported; NYHA = New York Heart Association.

	Male gender, %				DM, %				HT, %				Ischaemic heart disease, %				NYHA III/IV, %	
	HFNEF	*HFREF*	*LVH, no HF*	*No HF or LVH*	*HFNEF*	*HFREF*	*LVH, no HF*	*No HF or LVH*	*HFNEF*	*HFREF*	*LVH, no HF*	*No HF or LVH*	*HFNEF*	*HFREF*	*LVH, no HF*	*No HF or LVH*	*HFNEF*	*HFREF*
	81	72											87.9	84.1			16.7	22.9
	48	59.3		53.8													52	44
				38														
	53			76	12			7	100			100						
	66			63	13			0					13			0	34	
	61	81.1			40.2	38.2			70.1	57.1			50.6	83.4				
	48.6				30.5				80.4				53.7				73.0	
	47	82		45	18	24		0	100	88		13						
	45	63		52	27	30		11	53	48		29	66	83		14		
				48.1				14.7				43.5				16.5		
	52	0.64		60	33	32		30	48	31		50	17	50		70		
	43			40	51.47			30	66.17			60						
	36	83		34	30	37		0	93	91		1	35	52		0		
	46								100									
				48.3				14.8				53.1				19.5		
	50	66			29	28			51	40			30	44			47	59
	72	67		57	45.1	28.6		37.4	72.0	54.3		67.8	53.7	45.7		55.0	37	63
	39.9				52.8				83.5				18.8				39.4	
	52			26	29			36	69			78						
	41		45	30	31		16	9										
	54			55	19			14	100			100	38			30		
	34				34				96				21				83	
	58	86		46	40	49		3	88	69		36	32	59		0	16	37

In current clinical guidelines, the recommended natriuretic peptide cutoff values for the diagnosis of HF do not differ between HFREF and HFNEF.[1,2] However, there are some interesting differences in the epidemiology of natriuretic peptides between the HFREF and the HFNEF populations. Firstly, the increase of NT-proBNP levels is less pronounced in HFNEF.[6-8] NT-proBNP levels have been shown to be lower in HFNEF than HFREF patients of a similar NYHA class.[15] Furthermore, patients in the Irbesartan in heart Failure with Preserved Ejection Fraction Trial (I-PRESERVE) had low overall NT-proBNP levels.[16] These relatively lower natriuretic peptide levels suggest a lower diastolic wall stress in HFNEF when compared to HFREF. In case NT-proBNP is required to be elevated for a definite diagnosis of HF, only the higher-risk HFNEF patients will be identified, resulting in a reduced prevalence of HFNEF.[17] Secondly, the strategy of using elevated natriuretic peptides concentrations as a patient selection criterion for HFNEF trials could be questioned because the I-PRESERVE trial showed that the use of irbesartan was associated with improved outcomes in patients with NT-proBNP below, but not above, the median concentration.[16]

The above considerations suggest that natriuretic peptides may be less useful as a diagnostic and prognostic tool in HFNEF than in HFREF. Although NT-proBNP has a better negative predictive value than invasive measurement of left ventricular end-diastolic pressure, tissue doppler imaging indices and conventional echocardiography measurements in HFNEF patients, the overall diagnostic performance of NT-proBNP according to the receiver operating characteristic was only similar to that of tissue doppler imaging indices.[11] This calls for further exploration of other biomarkers which may provide incremental value specifically in HFNEF. The value of such other biomarkers will be discussed in this review.

Other biomarkers of myocyte stress

Another interesting biomarker of myocyte stress is adrenomedullin. Adrenomedullin is a hormone that lowers systemic vascular resistance and has natriuretic and diuretic effects. It is produced in several organs such as the heart, lungs and kidneys. We found one study that examined adrenomedullin in HFNEF (Table 4). Yu et al. showed that plasma adrenomedullin concentrations were higher in HFNEF patients than in healthy controls.[18] There was no significant difference in adrenomedullin concentration between HFNEF and HFREF patients. Furthermore, the authors concluded that adrenomedullin concentrations in patients with HF are especially raised in the presence of a restrictive filling pattern. The incremental value of adrenomedullin over natriuretic peptides has not yet been investigated.

BIOMARKERS OF INFLAMMATION

Pathophysiology

Biomarkers of inflammation are among the first to have been linked to HF. Early studies have focused on tumor necrosis factor α (TNF-α), interleukin-6 (IL-6) and C-reactive protein (CRP). These proinflammatory cytokines probably contribute to the clinical syndrome of HF and to progression of the disease through adverse effects on the vascular endothelium, myocyte apoptosis, induction of hypertrophy (e.g. by IL-6) and left ventricular dilatation (e.g. by TNF-α).[5] CRP has been correlated with the severity and prognosis of HF, as well as with response of HF patients to treatment.[19-22]

Inflammatory biomarkers may have different meanings in HFREF and HFNEF. In HFREF, inflammatory biomarkers may be a measure of heart failure severity. Although the source of cytokine production in HFREF is still unknown, it may possibly reside in the failing myocardium itself because of hemodynamic overload.[23] Alternatively, it may result from extramyocardial production in the bowel, because of altered tissue perfusion and tissue hypoxia, possibly modulated by bacterial endotoxin release from the gut.[23] In contrast, in the case of HFNEF, inflammatory biomarkers, particularly those associated with metabolic syndrome, may be a measure of risk for developing HFNEF. Obesity and diabetes mellitus are believed to play a major role in the remodeling of the ventricles and in the development of HFNEF.[24] Both obesity and diabetes mellitus are associated with increased inflammatory biomarker levels. As such, inflammatory biomarker levels may be a measure of factors driving left ventricular remodeling in HFNEF.

Moreover, a high body mass index appears to be beneficial in HFREF patients. Explanations for this obesity paradox in HFREF include possible protective effects conferred by excess body weight on HF mortality - because advanced HF is a catabolic state, obese patients with HF may have more metabolic reserve - and protective effects of cytokine and neuroendocrine profiles of obese patients.[25] However, this obesity paradox is missing in HFNEF,[24,26] which further underscores the important role of obesity in the development of this condition.

Biomarker levels in HFNEF

Although many studies have examined the associations of inflammatory biomarkers with HF in general, HFNEF has received less attention. Most of the studies that have included HFNEF patients have employed cross-sectional or case-control designs, and are summarized below (Table 2 and Supplemental Table 1).[19-22,27-31] Several studies have demonstrated elevated TNF-α and IL-6 levels in HFNEF patients when compared to a non-HF reference group.[19,22,28,29,31] Also, the concentrations of the TNF-α receptors

(sTNFR-1 and sTNFR-2) were found to be higher in HFNEF patients.[22, 28] Serum levels of CRP and Pentraxin-3 (PTX-3), a relatively newly-identified acute phase protein of the pentraxin superfamily which also includes CRP, were both found to be significantly higher in HFNEF patients when compared to with the non-HF reference group.[19-22] Finally, Collier et al. identified IL-8 and monocyte chemoattractant protein-1 (MCP-1) as novel inflammatory biomarkers of HFNEF.[31] These results once again emphasize that inflammatory biomarkers may play an important role in the development and progression of HFNEF.

Biomarker levels in HFNEF compared with HFREF

A few of the above mentioned studies also compared inflammatory marker concentrations in HFNEF with HFREF. Regarding this comparison, the results were less consistent between studies. Wisniacki et al. reported no significant difference in CRP, sTNFR2 and IL-6 levels between 25 HFNEF patients and 27 HFREF patients.[22] Michowitz et al. did not find a significant difference in CRP elevation between HFNEF and HFREF patients either.[20] Niethammer et al. showed that TNF-α and IL-10 were significantly elevated in HFREF but not in HFNEF.[28] Furthermore, sTNFR1 levels were less highly elevated in HFNEF than in HFREF, while sTNFR2 levels were similarly elevated in both groups. Matsubara et al. demonstrated less elevation of CRP, PTX3 and IL-6 levels in HFNEF compared to HFREF, while TNF-α concentrations were similarly elevated.[19] These findings suggest that several inflammatory biomarkers are more pronounced in HFREF, which supports the hypothesis that the origin and the meaning of these markers may differ between HFREF and HFNEF.

Association with incidence of HFNEF

Kalogeropoulos et al. examined 2610 persons aged 70-79 years from the Health ABC study and found that TNF-α (unadjusted HR per doubling 1.48; 95% CI 1.19-1.86) and IL-6 (unadjusted HR per doubling 1.81; 95% CI 1.23-2.68) were associated with incident HFNEF during follow-up.[32] Such associations of TNF-α and IL-6 were less strong for HFREF. CRP was not found to be a significant predictor. These findings support the hypothesis that inflammatory biomarkers are particularly associated with development of HFNEF.

Association with HFNEF prognosis

Few follow-up studies have been performed on the role of inflammatory biomarkers in HFNEF. Michowitz et al. found that CRP was independently associated with hospitalization in patients with HFREF, but not in patients with HFNEF.[20] These results support the hypothesis that inflammatory biomarkers are a measure of heart failure severity, particularly in HFREF. Mortality was not predicted by CRP levels in either patient category.

Within the Olmsted County study, Dunlay et al. performed a prospective study in which they examined 486 patients with active HF of whom 54% had HFNEF.[33] They found that mortality increased with increasing TNFα level (unadjusted HR of highest vs lowest quartile 2.10; 95% CI 1.30-3.38). No interaction was present between TNFα and ejection fraction, thus implying the effect was similar in HFNEF and HFREF.

BIOMARKERS OF EXTRACELLULAR-MATRIX REMODELING

Pathophysiology

The extracellular matrix provides a skeleton for myocytes and influences their size and shape.[5,32] Changes in the extracellular matrix may be causally related to remodeling of the ventricles resulting in progression of HF.[5] Collagen turnover in the extracellular matrix is mainly regulated by matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs). The MMPs are a family of endopeptidases that digest interstitial constituents.[30] The various MMPs have different substrates. TIMPs are proteins that bind to and inhibit the effects of MMPs. Furthermore, carboxy-terminal propeptide of procollagen type I (PICP), amino-terminal propeptide of procollagen type I (PINP) and type III (PIIINP) are markers of collagen biosysthesis, while carboxy-terminal telopeptide of collagen type I (CITP) is a marker of collagen degradation.

Histological research has demonstrated that the nature of fibrosis differs between HFNEF and HFREF.[34] Fibrosis in HFNEF is mainly interstitial, while fibrosis in HFREF is both interstitial and replacement fibrosis.[34] Therefore, it may be expected that different pathophysiological pathways are activated and that different profiles of collagen biomarkers are expressed in HFNEF versus HFREF. Previous studies have shown that interstitial fibrosis is associated with increased expression of TIMPs,[35] while replacement fibrosis is associated with increased expression of MMPs.[36] As such, it may be expected that TIMPs are particularly upregulated in HFNEF, while several MMPs are particularly upregulated in HFREF. Furthermore, in contrast to HFREF, myocardial stiffness may even be more important than extracellular fibrosis as a mechanism for diastolic stiffness in HFNEF.[37] HFNEF patients with only mild elevations of collagen volume fraction may have highly elevated left ventricular end-diastolic pressures.[38] Unfortunately, no biomarkers have been identified yet in order to measure this myocardial stiffness.

Biomarker levels in HFNEF

Most studies have applied a cross-sectional or case-control design (Table 3 and Supplemental table 2).[30,31,39-43] Although some of the results were not consistent between studies, most of the studies showed that MMP-1, MMP-2, MMP-9, TIMP-1, PICP, PIIINP and CITP were elevated in HFNEF patients when compared to a control

TABLE 2. Biomarkers of inflammation.

	Index group (n)	*Reference group (n)*	*Outcome*	CRP	PTX-3	TNF-α	sTNFR1	sTNFR2	IL-6	Cardio-trophin-1	IL-8	IL-10	MCP-1
Cross-sectional / case-control studies comparing HFNEF with a non-HF reference group													
Amosova et al. (23)	hospitalized HFNEF (26)	healthy (10)	level			↑			↑				
Wisniacki et al. (19)	outpatient HFNEF (25)	healthy (26)	level	↑				↑	↑				
Michowitz et al. (17)	outpatient HFNEF (77)	healthy (7701)	level	↑									
Niethammer et al. (24)	hospitalized HFNEF (17)	healthy (20)	level			ns	↑	↑	↑			ns	
Okuyan et al. (18)	hospitalized HFNEF (68)	healthy (40)	level	↑									
Matsubara et al. (16)	hospitalized HFNEF (82)	non-HF patients (171)	level	ns	↑	↑			↑				
Wu et al. (25)	hospitalized HFNEF (110)	non-HF patients (50)	level			↑			↑				
Zile et al. (26)	outpatient HFNEF (61)	healthy (241)	level							ns			
	outpatient HFNEF (61)	LVH without HF (144)	level							ns			
Collier et al. (27)	outpatient HFNEF (181)	HT patients without HF (94)	level			ns			↑		↑		↑
Cross-sectional / case-control studies comparing HFNEF with HFREF													
Wisniacki et al. (19)	outpatient HFNEF (25)	outpatient HFREF (27)	level	ns				ns	ns				
Michowitz et al. (17)	outpatient HFNEF (77)	outpatient HFREF (217)	level	ns									
Niethammer et al. (24)	hospitalized HFNEF (17)	hospitalized HFREF (17)	level			↓	↓	ns	ns			↓	
Matsubara et al. (16)	hospitalized HFNEF (82)	hospitalized HFREF (70)	level	↓	↓	ns			↓				

TABLE 2 continued.

	Index group (n)	*Reference group (n)*	*Outcome*	CRP	PTX-3	TNF-α	sTNFR1	sTNFR2	IL-6	Cardio-trophin-1	IL-8	IL-10	MCP-1
Follow-up studies													
Michowitz et al. (17)	outpatient HFNEF (77)		Hospitalization (2.8y follow-up)	ns									
			Mortality (2.8y follow-up)	ns									
Dunlay et al. (29)	community-based HF patients* (486)		Mortality (1y follow-up)			HR 2.10 (1.30-3.38)†							
Kalogeropoulos et al. (28)	community-based aged 70-79 (2610)		HFNEF (9.4y follow-up)	ns		HR 1.81 (1.23-2.68)‡			HR 1.48 (1.19-1.86)‡				

↑ Indicates a significantly higher biomarker level in the index group compared to the reference group. ↓ Indicates a significantly lower biomarker level in the index group compared to the reference group. HF = heart failure; HFNEF = heart failure with normal ejection fraction; HFREF = heart failure with reduced ejection fraction; CRP = C-reactive protein; HT = hypertension; IL = interleukin; LVF = left ventricular hypertrophy; MCP-1 = monocyte chemoattractant protein 1; ns = not significant; PTX-3 = pentraxin 3; TNF-α = tumor necrosis factor α; sTNFR = soluble tumor necrosis factor receptor.

* Of whom 54.8% had HFNEF. † Unadjusted hazard ratio of highest quartile compared to lowest quartile. ‡ Unadjusted hazard ratio per doubling.

group consisting of patients without HF.[30,31,39-43] Also MMP-3, MMP-7, TIMP-4 and Osteopontin concentrations might be elevated in HFNEF, but these biomarkers are less well investigated.[30] Interestingly, lower serum concentrations of MMP-8 and MMP-13 were observed in patients with HFNEF.[30,39] Furthermore, Gonzalez et al. observed that the MMP-1/TIMP-1 ratio was increased in HFNEF patients with normal left-sided filling pressures.[43] These findings support the hypothesis that the balance of collagen turnover is disturbed in patients with HFNEF.

Biomarker levels in HFNEF compared with HFREF

Only two studies compared biomarker levels of HFNEF with HFREF patients. Frantz et al. reported similarly elevated TIMP-1 levels in hospitalized HFNEF and HFREF patients. Naito et al. found similarly elevated plasma concentration of MMP-2 in hospitalized HFNEF and HFREF patients. MMP-1 concentration, however, was significantly higher in HFREF patients. The observed difference in MMP-1 concentration supports the hypothesis that different profiles of extracellular-matrix biomarkers are expressed in HFREF and HFNEF.

Association with incidence of HFNEF

Barasch et al. performed a nested case-control study within the Cardiovascular Health study, which has longitudinal follow-up.[44] Biomarkers were assessed at 5-year or 9-year follow-up in a total of 880 subjects (131 with systolic HF, 179 with HFNEF, 280 controls with cardiovascular risk factors and 279 healthy controls). In the total study population, elevated CITP (OR per tertile 3.1; 95% CI 2.4 to 4.0) and PIIINP (OR per tertile 2.2; 95% CI 1.7 to 2.8) were associated with incident HFNEF during follow-up.

Association with HFNEF prognosis

Follow-up studies on the prognostic utility of biomarkers of extracellular matrix remodeling are also small in number. Within the Irbesartan in Heart Failure With Preserved Systolic Function (I-PRESERVE) trial, Krum et al. investigated the prognostic value of PINP, PICP and Osteopontin during follow-up of 4.1 years.[45] In univariable analysis, increased levels of these biomarkers were all associated with the composite endpoint of mortality and hospitalization, all-cause mortality and the composite of HF related death or hospitalization. However, none of these biomarkers remained significant as an independent predictor when introduced into a multivariable model adjusting for 19 clinical parameters. These results are in line with above-mentioned findings suggesting that myocardial stiffness is a more important factor of diastolic dysfunction than fibrosis in patients with HFNEF.

OTHER BIOMARKERS

Homocysteine

Homocysteine is traditionally believed to have pro-oxidative, pro-inflammatory and vasoconstrictive properties, and to cause endothelial vascular dysfunction. Experimental studies have demonstrated that elevated homocysteine levels may also adversely affect the myocardium, leading to pathological hypertrophy of ventricles with disproportionate increase in collagen.[21] Okuyan et al. measured homocysteine concentrations in 68 hospitalized HFNEF patients and 40 healthy controls (Table 4 and Supplemental table 3).[21] Homocysteine concentrations were significantly higher in HFNEF patients. The authors state that pathologic mechanisms and effects of homocysteine on the natural history of HF still need to be clarified.

Growth differentiation factor 15

Growth differentiation factor 15 (GDF-15) is suggested to be a downstream marker indicative of different pathways of myocardial stress and inflammation. In animal models, GDF-15 was found to attenuate reduction in fractional shortening and to protect the heart from hypertrophy and ischemia-reperfusion injury.[46] Stahrenberg et al. measured GDF-15 in 142 HFNEF patients, 86 HFREF patients and 188 healthy elderly controls.[46] They found that GDF-15 was higher in HFNEF compared to controls. Serum GDF-15 concentrations were equal in HFNEF and HFREF patients. The authors concluded that diagnostic precision of GDF-15 was at least as good as that of NT-proBNP, and that a combination significantly improved diagnostic accuracy. Similar results were reported by Santhanakrishnan et al.[47]

Cystatin C

Renal function is also believed to play a role in the evolvement of HF.[48, 49] Cystatin C is a marker of renal function. Moran et al examined 4453 subjects aged 65 years or older without HF at baseline from the Cardiovascular Health Study.[48] They compared the association of cystatin C with risk of incident HFNEF and HFREF. During 8 years of follow-up, 167 cases of incident HFNEF and 206 cases of incident HFREF occurred. Increased risk of HFNEF was apparent only in the highest cystatin C quartile (HR 2.25; 95% CI 1.33-3.80), while a linear trend was present for HFREF. This study demonstrates that kidney dysfunction is a risk factor for occurrence and progression of HF. Carrasco-Sanchez et al. investigated the prognostic value of cystatin C in HFNEF.[49] They included 218 hospitalized HFNEF patients and collected 1-year follow-up. Cystatin C was a strong predictor of the composite of mortality or hospitalization (HR of highest compared to lowest quartile 4.85; 95% CI 2.76-8.51) and mortality alone (HR 11.35; 95% CI 4.01-32.14). Cystatin C also remained a strong independent predictor with multivariable analysis.

Table 3. Biomarkers of extracellular matrix remodeling.

	Index group (n)	*Reference group (n)*	*Outcome*	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8
Cross-sectional / case-control studies comparing HFNEF with a non-HF reference group								
Ahmed et al. (35)	outpatient HFNEF (26)	healthy without HT (39)	level		ns			
	outpatient HFNEF (26)	healthy with HT (14)	level		ns			
	outpatient HFNEF (26)	LVH without HF (23)	level		ns			
Martos et al. (36)	outpatient HFNEF (32)	HT patients without HF (54)	level	ns	↑			
Frantz et al. (37)	hospitalized HFNEF (102)	healthy (74)	level					
Naito et al. (38)	hospitalized HFNEF (42)	non-HF patients (10)	level	↑	↑			
Gonzales et al. (39)	outpatient HFNEF (156)	healthy (20)	level	↑				
Zile et al. (26)	outpatient HFNEF (61)	healthy (241)	level	ns	↑	ns	↑	ns
	outpatient HFNEF (61)	LVH without HF (144)	level	ns	↑	↑	ns	↓
Collier et al. (27)	outpatient HFNEF (181)	HT patients without HF (94)	level		↑			
Cross-sectional / case-control studies comparing HFNEF with HFREF								
Frantz et al. (37)	hospitalized HFNEF (102)	hospitalized HFREF (147)	level					
Naito et al. (38)	hospitalized HFNEF (42)	hospitalized HFREF (68)	level	↓	ns			
Follow-up studies								
Barasch et al. (40)	community-based aged ≥65 (880)		HFNEF (4y follow-up)					
Krum et al. (41)	outpatient HFNEF aged ≥60 (313)		mortality or hospitalization (4.1y follow-up)					
			all-cause mortality (4.1y follow-up)					
			HF death or hospitalization (4.1y follow-up)					

↑ Indicates a significantly higher biomarker level in the index group compared to the reference group. ↓ Indicates a significantly lower biomarker level in the index group compared to the reference group. CITP = carboxy-terminal telopeptide of collagen type I; HF = heart failure; HFNEF = heart failure with normal ejection fraction; HFREF = heart failure with reduced ejection fraction; HT = hypertension; LVF = left ventricular hypertrophy; MMP = matrix metalloproteinasis; ns = not significant; PICP = carboxy-terminal propeptide of procollagen type I; PIIINP = amino-terminal propeptide of procollagen type III; PINP = amino-terminal propeptide of procollagen type I; TIMP = tissue inhibitor of metalloproteinasis.
* Unadjusted odds ratio per tertile.
† Unadjusted hazard ratio per 10 µg/L.
‡ Unadjusted hazard ratio per 10 nmol/L.

	MMP-9	MMP-13	TIMP-1	TIMP-2	TIMP-3	TIMP-4	PINP	PICP	PIIINP	CITP	Osteopontin
	↑	↓	↑	ns							
	↑	↓	↑	ns							
	ns	ns	↑	ns							
	↑		ns				ns	↑	↑	↑	
	ns		↑								
			↑					↑			
	↑		↑	↑	ns	↑	ns		↑	↑	↑
	ns		ns	ns	ns	↑	ns		↑	ns	ns
	↑		ns				ns	ns	↑	↑	
			ns								
								ns	OR 2.2 (1.7-2.8)*	OR 3.1 (2.4-4.0)*	
							HR 1.09 (1.05-1.13)†		HR 2.47 (0.97-6.33)†		HR 1.08 (1.03-1.15)‡
							HR 1.06 (1.03-1.09)†		HR 2.85 (1.52-5.36)†		HR 1.06 (1.02-1.11)‡
							HR 1.09 (1.05-1.13)†		HR 5.91 (2.94-11.88)†		HR 1.06 (0.99-1.14)‡

Table 4. Other biomarkers.

	Index group (n)	*Reference group (n)*	*Outcome*	Adreno-medullin	Nor-adrenalin
Cross-sectional / case-control studies comparing HFNEF with a non-HF reference group					
Yu et al. (15)	hospitalized HFNEF (31)	healthy (17)	level	↑	
Wisniacki et al. (19)	oupatient HFNEF (25)	healthy (26)	level		ns
Varol et al. (49)	outpatient HFNEF (32)	healthy (30)	level		
Okuyan et al. (18)	hospitalized HFNEF (68)	healthy (40)	level		
Stahrenberg et al. (42)	community-based HFNEF (142)	healthy (188)	level		
Zile et al. (26)	outpatient HFNEF (61)	healthy (241)	level		
	outpatient HFNEF (61)	LVH without HF (144)	level		
Santhanakrishnan et al. (43)	in- and outpatient HFNEF (50)	healthy (50)	level		
Cross-sectional / case-control studies comparing HFNEF with HFREF					
Yu et al. (15)	hospitalized HFNEF (31)	hospitalized HFREF (46)	level	ns	
Wisniacki et al. (19)	outpatient HFNEF (25)	outpatient HFREF (27)	level		ns
Stahrenberg et al. (42)	community-based HFNEF (142)	community-based HFREF (86)	level		
De Boer et al. (13)	hospitalized HFNEF (114)	hospitalized HFREF (368)	level		
Santhanakrishnan et al. (43)	in- and outpatient HFREF (51)	in- and outpatient HFREF (51)	level		
Follow-up studies					
Moran et al. (44)	community-based aged ≥65 (4453)		HFNEF (8y follow-up)		
Butler et al. (46)	community-based aged 70-79 (2902)		HFNEF (9.4y follow-up)		
De Boer et al. (13)	hospitalized HF (592)		mortality or hospitalization (1.5y follow-up)		
Carrasco-Sanchez et al. (45)	hospitalized HFNEF (218)		mortality or hospitalization (1y follow-up)		
			all-cause mortality (1y follow-up)		

↑ Indicates a significantly higher biomarker level in the index group compared to the reference group.
↓ Indicates a significantly lower biomarker level in the index group compared to the reference group.
CA-125 = Carbohydrate antigen 125; GDF-15 = growth differentiation factor 15; HF = heart failure; HFNEF = heart failure with normal ejection fraction; HFREF = heart failure with reduced ejection fraction; LVF = left ventricular hypertrophy; ns = not significant; sRAGE = soluble receptor for advanced glycation end-product.
* Hazard ratio of highest quartile compared to lowest quartile adjusted for age, gender and race.
† Unadjusted hazard ratio per 10ng/mL.
‡ Unadjusted hazard ratio per doubling.
§ Unadjusted hazard ratio of highest quartile compared to lowest quartile.

	Homo-cysteine	GDF-15	Cystatin C	Resistin	Galectin-3	sRAGE	CA-125	Troponin-T	ST2
							ns		
	↑								
		↑							
						ns			
						ns			
		↑						↑	↑
		ns							
					ns				
		ns						↓	ns
			HR 2.25 (1.33-3.80)*						
				HR 1.42 (1.27-1.58)†					
					HR 1.97 (1.62-2.42)‡				
			HR 4.85 (2.76-8.51)§						
			HR 11.35 (4.01-32.14)§						

Resistin

Another potentially interesting biomarker is resistin. Resistin has been found to be produced and released from adipose tissue. Although the exact function of resistin is not known, it has been associated with insulin resistance and inflammatory response.[49, 50] Resistin concentrations have previously been correlated with risk of coronary artery disease, renal dysfunction and adverse outcomes among stroke patients.[50] Butler et al. measured resistin in 2902 subjects aged 70-79 years without prevalent HF from the health ABC study, where after these patients were followed-up for incident HF.[50] Resistin was found to be associated with both incident HFNEF (HR per 10ng/mL 1.42; 95% CI 1.27-1.58) and incident HFREF (HR per 10ng/mL 1.35; 95% CI 1.20-1.53). The prognostic value of resistin in HFNEF patients has not been investigated yet. Another study showed that leptin, which is also a biomarker related to adiposity and metabolic syndrome, is associated with incidence of HF.[51] A third biomarker in this category, adiponectin, was not found to be associated with HF.[52] Overall, these findings are in line with the hypothesis that obesity and the metabolic syndrome drive development of HF.

Galectin-3

Galectin-3 is a protein that has a broad biological functionality. It is known to be involved in cell adhesion, cell activation, chemo-attraction, cell growth, cell differentiation, fibroblast activation and apoptosis.[15, 52] Galectin-3 has been proposed as a novel biomarker of HF. It was found to be associated with increased risk for incident HF and mortality.[15, 53] De Boer et al. showed that galectin-3 levels did not differ between 114 hospitalized HFNEF patients and 368 HFPEF patients.[15] In the overall HF study population, galectin-3 was found to be a significant predictor of the composite of mortality and hospitalization (HR per doubling 1.97; 95% CI 1.62-2.42). The predictive value of galectin-3 was stronger in HFNEF compared to HFREF. Furthermore, combined galectin-3 and BNP levels increased prognostic value over either biomarker alone.

Advanced glycation end-products

Advanced glycation end-products (AGEs) are formed through a reaction between proteins and sugar residues. AGEs and their soluble receptors (sRAGEs) are known to induce intracellular damage and to play a role in chronic inflammation.[30] Enhanced accumulation of AGE is thought to play a role in the pathophysiology of chronic HF. Zile et al. measured sRAGE concentration in HFNEF patients and controls. They could not detect a significant sRAGE elevation in HFNEF.[30]

Carbohydrate antigen 125

Carbohydrate antigen 125 (CA-125) is traditionally known as a tumor marker for

ovarian cancer. However, non-malignant serosal effusions may display elevated serum CA125 levels as well, most likely due to increased CA-125 production by the serosal mesothelium.[53] Previously, CA125 has been shown to be elevated in HF and to be related with HF severity.[53] Varol et al. measured CA-125 concentrations in 32 HFNEF outpatients with hypertrophic cardiomyopathy and in 30 healthy controls.[53] Although the difference in CA-125 level between the groups was not significant, CA-125 levels increased with NYHA class and level of diastolic dysfunction.

Troponin-T

Troponin-T is a well-established marker of myocardial necrosis in acute coronary syndromes, but its role in HF is less well defined.[47] Santhanakrishnan et al. have evaluated several emerging biomarkers, including high sensitivity troponin-T.[47] They found that troponin-T levels were higher in HF patients compared to healthy control subjects. Furthermore, troponin-T concentration was higher in HFREF than in HFNEF, even after adjusting for clinical covariates, which suggests that myocyte injury is higher in HFREF.

ST2

ST2 is a member of the interleukin-1 receptor family and is involved in the process of ventricular remodeling.[47] ST2 may be upregulated in cardiac myocytes and fibroblasts subjected to mechanical stress. Santhanakrishnan et al. found that HFNEF patients had higher serum levels of ST2 compared to healthy control subjects.[47] However, this difference did not remain statistically significant after adjustment for age, sex and clinical covariates. Furthermore, there was no difference in ST2 concentration between HFREF and HFNEF patients. Nevertheless, previous studies have demonstrated that ST2 was an independent predictor of mortality in patients with acute HF and that ST2 was equally predictive in patients with HFREF and HFNEF.[54]

Discussion

Although a significant body of research has been generated in the past decade on the role of biomarkers in HF, the majority of prognostic studies have included HFREF patients. Studies on the prognostic and incremental value of biomarkers, other than BNP and NT-proBNP, in HFNEF are scarce or lacking. Most of the studies on biomarkers in HFNEF patients are cross-sectional in design and have included limited numbers of patients. Only a few prospective studies have been conducted. To the best of our knowledge, this is the first review that specifically focuses on the role of biomarkers in HFNEF.

Biomarkers examined most extensively in HFNEF include biomarkers of myocyte stress, inflammation and extra-cellular matrix remodeling. Some of these biomarkers have been shown to be increased to a different extent in HFNEF and HFREF.[19,28,42] In general, the degree of marker expression in the failing myocardium is likely to depend on the type, degree, and duration of the specific extracellular stimuli.[51] As described above, inflammatory biomarkers may have different meanings in HFNEF and HFREF. Obesity and the metabolic syndrome, associated with increased concentration of several inflammatory and metabolic markers, may drive development of HF, particularly in HFNEF.[24] On the other hand, inflammatory biomarkers may be a measure of severity of HF, particularly in HFREF.[23] Furthermore, fibrosis and myocardial stiffness occur to a different extent and have a different nature in HFNEF versus HFREF.[34] In HFNEF, myocyte stiffness may play a more important role than myocardial fibrosis.[37] This may be reflected by differences in levels of biomarkers of extracellular-matrix remodeling between HFNEF and HFREF.

Specifically, HFREF, typically characterized by volume overload, left ventricular dilatation, eccentric left ventricular remodeling and low relative wall thickness, can particularly be expected to display upregulation of biomarkers such as NT-proBNP (myocyte stress), GDF-15 (stress pathway), TNF-**α** (stimulating MMP expression), several MMPs (proteolytic enzymes) and CITP (collagen degradation).[28,46] On the other hand, HFNEF, characterized more often by a non-dilated left ventricle, concentric left ventricular hypertrophy and probably driven by metabolic syndrome, may particularly be associated with upregulation of biomarkers such as TIMPs (inhibiting collagen proteolysis), Galectin-3 (fibroblast activation), PINP, PIIINP (collagen biosynthesis), homocysteine (associated with hypertrophy) and resistin (adipose tissue and insulin resistance).[15, 21, 50] In this review, we have observed that levels of several biomarkers of inflammation, including CRP, PTX-3, TNF-**α**, IL-6 and IL-10, are higher in HFREF compared to HFNEF, although some of these results were not consistent between studies. Together with previous data on MMP and TIMP levels in several animal models, these observations provide some support for the above-mentioned hypothesis regarding the differences between biomarker patterns in HFNEF and HFREF.[51]

Based on the present review, several biomarkers and biomarker categories, including biomarkers of myocyte stress, inflammation, extracellular matrix remodeling, GDF-15, cystatin C, resistin and galectin-3, appear to be potentially promising diagnostic tools in HFNEF. Some of them, including TNF-**α**, IL-6, PINP, PIIINP, osteopontin and cystatin C, may carry prognostic value as well. Further research may provide additional evidence for the value of these biomarkers in improvement of risk stratification of patients with

HFNEF. Furthermore, the balance between various markers wintin the same category (e.g. MMP/TIMP ratios and balance between inhibitory and stimulatory cytokines) may also have diagnostic and prognostic value, and should be further investigated as well. Applying a multiple-biomarker strategy may result in even further improvement of risk stratification compared to using one biomarker alone.[15,30] For example, a multiple-biomarker panel consisting of increased MMP-2, TIMP-4, PIIINP, and decreased MMP-8 was able to identify HFNEF patients with an area under the receiver operating characteristic curve of 0.79, which was better than any single biomarker, including NT-proBNP, or clinical covariates alone.[30] Such results are promising and should be further investigated before such biomarkers can be used in clinical practice.

Some of the studies we reviewed displayed inconsistent results. This may in part be explained by the lack of power to detect significant differences in biomarkers levels and clinical outcomes, as sample size was modest in many of the studies. Furthermore, various definitions of HFNEF were used by the individual studies, and the LVEF cutoff value ranged from ≥40% to ≥55%. Moreover, large variations were present in the choice of the reference groups.

Future directions

Currently, natriuretic peptides are the only biomarkers routinely used for diagnosis and risk stratification in common clinical practice.[1, 2] Before other known biomarkers may be used, their incremental diagnostic and prognostic value over established clinical covariates and imaging techniques, such as echocardiography, should be evaluated. Preferably, this should be done in an epidemiological setting using a gold standard, including measures of cardiac structure and function in addition to clinical presentation, to demonstrate the diagnostic power of a specific biomarker. Moreover, as mentioned above, a multiple-biomarker panel may have higher diagnostic and prognostic value than any single biomarker alone. Therefore, large, prospective studies measuring multiple biomarkers are urgently needed in order to further elucidate the role and value of biomarkers in HF in general. With regard to the diagnostic and prognostic value of biomarkers in HFNEF in particular, evidence is much less abundant so far and studies have again mainly focused on natriuretic peptides. The other, most promising, biomarkers pertaining specifically to HFNEF at the moment include markers of collagen turnover and collagen signaling pathways.

Meanwhile, it is likely that ongoing fundamental and epidemiologic research will also yield new classes of potentially useful HF biomarkers. Several, relatively novel research techniques, such as genomics and proteomics, are promising contributors to biomarker

discovery. New biomarkers may contribute to further improvement of prognostication and may improve our understanding of the complex pathophysiology of HF as well. Moreover, by comparing biomarker patterns and their prognostic value between patients with reduced and normal LVEF, further etiologic insights into the development of HFNEF may be obtained.

Conflicts of interest

None declared.

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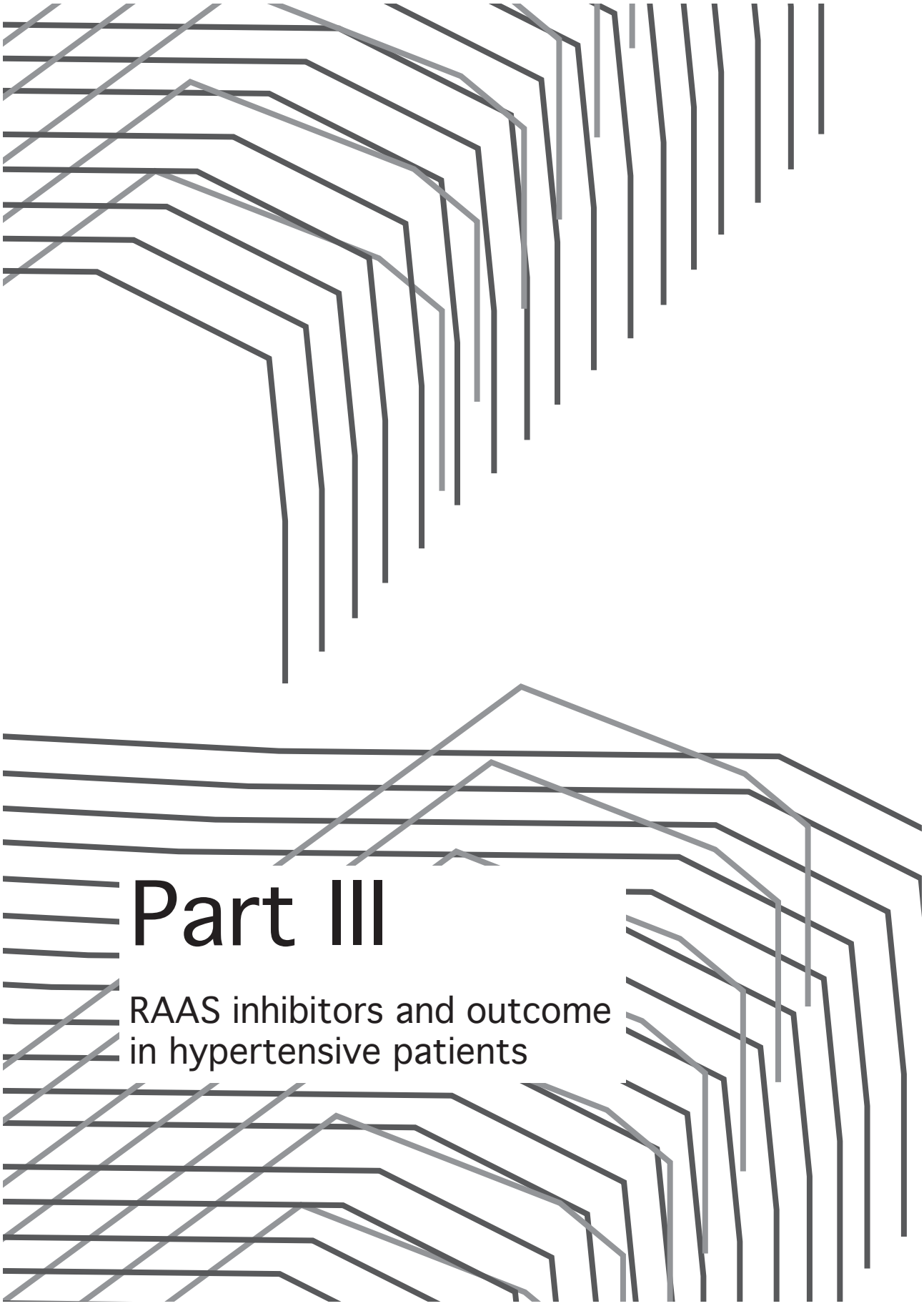
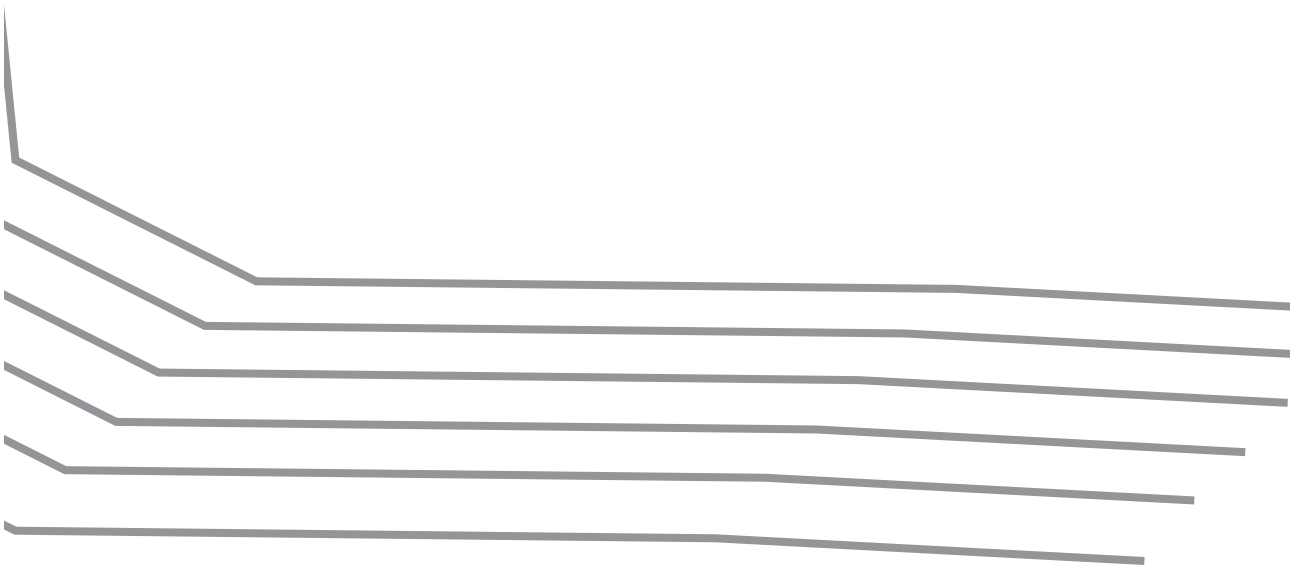


Part III

RAAS inhibitors and outcome in hypertensive patients



Chapter 11

Angiotensin-converting enzyme inhibitors reduce mortality in hypertension: a meta-analysis of randomized clinical trials of renin-angiotensin-aldosterone system inhibitors involving 158 998 patients

L.C. van Vark, M. Bertrand, K.M. Akkerhuis, J.J. Brugts, K. Fox, J.J. Mourad, E. Boersma

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Abstract

Aims

Renin–angiotensin–aldosterone system (RAAS) inhibitors are well established for the reduction in cardiovascular morbidity, but their impact on all-cause mortality in hypertensive patients is uncertain. Our objective was to analyse the effects of RAAS inhibitors as a class of drugs, as well as of angiotensin-converting enzyme (ACE) inhibitors and AT1 receptor blockers (ARBs) separately, on all-cause mortality.

Methods and Results

We performed a pooled analysis of 20 cardiovascular morbidity–mortality trials. In each trial at least two-thirds of the patients had to be diagnosed with hypertension, according to the trial-specific definition, and randomized to treatment with an RAAS inhibitor or control treatment. The cohort included 158 998 patients (71 401 RAAS inhibitor; 87 597 control). The incidence of all-cause death was 20.9 and 23.3 per 1000 patient-years in patients randomized to RAAS inhibition and controls, respectively. Overall, RAAS inhibition was associated with a 5% reduction in all-cause mortality (HR: 0.95, 95% CI: 0.91–1.00, P= 0.032), and a 7% reduction in cardiovascular mortality (HR: 0.93, 95% CI: 0.88–0.99, P= 0.018). The observed treatment effect resulted entirely from the class of ACE inhibitors, which were associated with a significant 10% reduction in all-cause mortality (HR: 0.90, 95% CI: 0.84 – 0.97, P= 0.004), whereas no mortality reduction could be demonstrated with ARB treatment (HR: 0.99, 95% CI: 0.94 – 1.04, P= 0.683). This difference in treatment effect between ACE inhibitors and ARBs on all-cause mortality was statistically significant (P-value for heterogeneity 0.036).

Conclusion

In patients with hypertension, treatment with an ACE inhibitor results in a significant further reduction in all-cause mortality. Because of the high prevalence of hypertension, the widespread use of ACE inhibitors may result in an important gain in lives saved.

Introduction

The World Health Organization describes hypertension as the number one risk factor for mortality, as worldwide annually 7.5 million deaths (13% of all deaths) are attributable to high blood pressure (BP)-related diseases, particularly cardiovascular diseases (CVD).[1] For that reason, the guidelines of hypertension and cardiology societies emphasize that hypertension treatment should aim at reducing the long-term risk of (cardiovascular) morbidity and mortality.[2,3] Hypertension is often referred to as the 'silent killer', as its presence is usually symptomless. Therefore, compliance to antihypertensive medication is a challenge for most patients, especially as adequate BP control often requires the use of multiple agents, causing additional side effects and as a result inferior adherence.[2] Thus, there is a continuing need for potent medications, preferably with beneficial effects on mortality, to improve patients' adherence to the treatment prescribed.

The benefits of antihypertensive treatment on cardiovascular morbidity are thought to be mainly due to the BP-lowering effect *per se*, independent of the class of drug employed, as has been demonstrated with β-blockers, diuretics, calcium channel blockers, and recently with the renin–angiotensin–aldosterone system (RAAS) inhibitors.[2] Blockade of the RAAS is one of the key therapeutic targets in patients with hypertension, as an overactive RAAS is strongly associated with high BP. The RAAS controls circulating volume and electrolyte balance in the human body and is therefore an important regulator of haemodynamic stability.[4] RAAS inhibitors are the most widely prescribed class of drugs for the management of hypertension. Currently, the most clinically relevant pharmacological agents that block the RAAS are angiotensin-converting enzyme (ACE) inhibitors and AT1 receptor blockers (ARBs). Both drugs block angiotensin II, but ACE inhibitors are characterized by a decrease in the degradation of bradykinin leading to a release of nitric oxide and prostaglandins resulting in additional vasodilatation. These differences in modes of action between ACE inhibitors and ARBs might have clinical implications for patients with hypertension.[5]

Reductions in both cardiovascular morbidity and mortality have been well demonstrated with RAAS inhibitors across specific populations that were selected and included for a criterion other than hypertension *per se*. For example, SOLVD (enalapril in heart failure), HOPE (ramipril in patients with high CVD risk), and EUROPA (perindopril in stable coronary disease) demonstrated significant reductions in the composite endpoint of death from cardiovascular causes, myocardial infarction or stroke with ACE inhibitors. In these trials, less than half of the patients enrolled had prevalent hypertension.[6-8] The beneficial effects of RAAS inhibitors on (all-cause) mortality (a guideline-recommended goal of

antihypertensive therapy)[2] have not been convincingly demonstrated in the indication of hypertension. Furthermore, most (antihypertensive) trials in which the clinical effects of RAAS inhibitors were evaluated were underpowered for this endpoint.[9-11] To evaluate the impact of RAAS inhibitors on all-cause and cardiovascular mortality for their main indication, hypertension, we undertook a meta-analysis of all prospective randomized clinical trials that compared RAAS inhibitors with control therapy in different populations in which the absolute majority of the patients had hypertension, and where the expected benefits would mainly come from a decrease in BP.

We hypothesized that, taken all evidence together, RAAS inhibitors would produce a significant mortality reduction compared with (contemporary) control therapy. Although the primary aim of this meta-analysis decided a priori was to evaluate RAAS inhibitors as a class of drugs, we realized that ACE inhibitors and ARBs have partly different modes of action. Therefore, we decided to also study these two classes of drugs separately.

We argued that, if a significant effect on both all-cause and cardiovascular mortality could be demonstrated, then treating physicians would have an additional argument to motivate hypertensive patients to comply with long-term treatment with these agents.

Methods

Study Selection

We intended to include all publicly available morbidity-mortality prospective randomized controlled trials that compared active treatment with an ACE inhibitor or an ARB with control treatment (placebo, active control or usual care).

Trials were identified by a systematic search of OVID MEDLINE and (ADIS) ISI Web of Science using a broad range of key words, including 'antihypertensive agents', 'angiotensin-converting enzyme inhibitors', 'angiotensin II Type 1 receptor blockers', 'hypertension', and 'mortality', published in English between January 1st, 2000 and March 1st, 2011. We decided to start our search in the year 2000, because of our intention to evaluate the effect of RAAS inhibition on top of contemporary treatment and considered the HOPE trial to be a landmark study in this respect (published in the year 2000).[7] References of identified papers and abstract listings of annual meetings of the American Heart Association, the American College of Cardiology, European Society of Cardiology, the American Society of Hypertension, the European Society of Hypertension, and the Council for High Blood Pressure Research were also examined during the same period.

Each trial identified in this search was critically and independently evaluated by two investigators (L.v.V. and K.M.A.) for patient population, study treatment, protocol, and endpoints.

A total of 512 publications met the above-mentioned search criteria (Figure1). We selected trials including different hypertensive populations for whom the benefits of RAAS inhibition would be expected to be mainly due to BP reduction. We only included the principal study publication, and excluded *post hoc* and subgroup analyses. Furthermore, we excluded trials in which patients were selected because of a specific disease, such as heart failure, acute coronary syndromes, acute stroke, haemodialysis, atrial fibrillation, or post-cardiac surgery patients, because of the expected benefits of RAAS inhibition beyond BP lowering in these patient populations.[12, 13]

Forty-four randomized controlled trials using RAAS blockade were identified that corresponded with the inclusion criteria. We additionally excluded eight trials in which less than two-thirds (66·7%) of the studied population were diagnosed with hypertension, according to the trial-specific definition. Ten trials were excluded due to either a low number of participants (n <100) or a low incidence of all-cause death (n <10), the primary endpoint of this study. Moreover, one trial was excluded because all-cause mortality was not reported. Finally, five trials (including INVEST, ACCOMPLISH, and ONTARGET) were excluded because RAAS inhibitors were used simultaneously in both trial arms.[14-16] Thus, a total of 20 trials were included in our analysis (Figure1), which had a follow-up duration of at least 1 year.

Data Extraction

This analysis is based on data that were obtained from the trials' main results papers. Two authors (LvV, KMA) independently extracted data from these reports, and resolved differences by consensus. For each treatment arm, we recorded the number of trial participants, the number of patients who reached the endpoint of all-cause and cardiovascular mortality, the mean age at baseline, the mean diastolic and systolic blood pressure (SBP) at baseline, the percentage of male participants, the percentage of patients with diabetes mellitus, renal insufficiency and hypertension, as well as the total follow-up time (until death) in years.

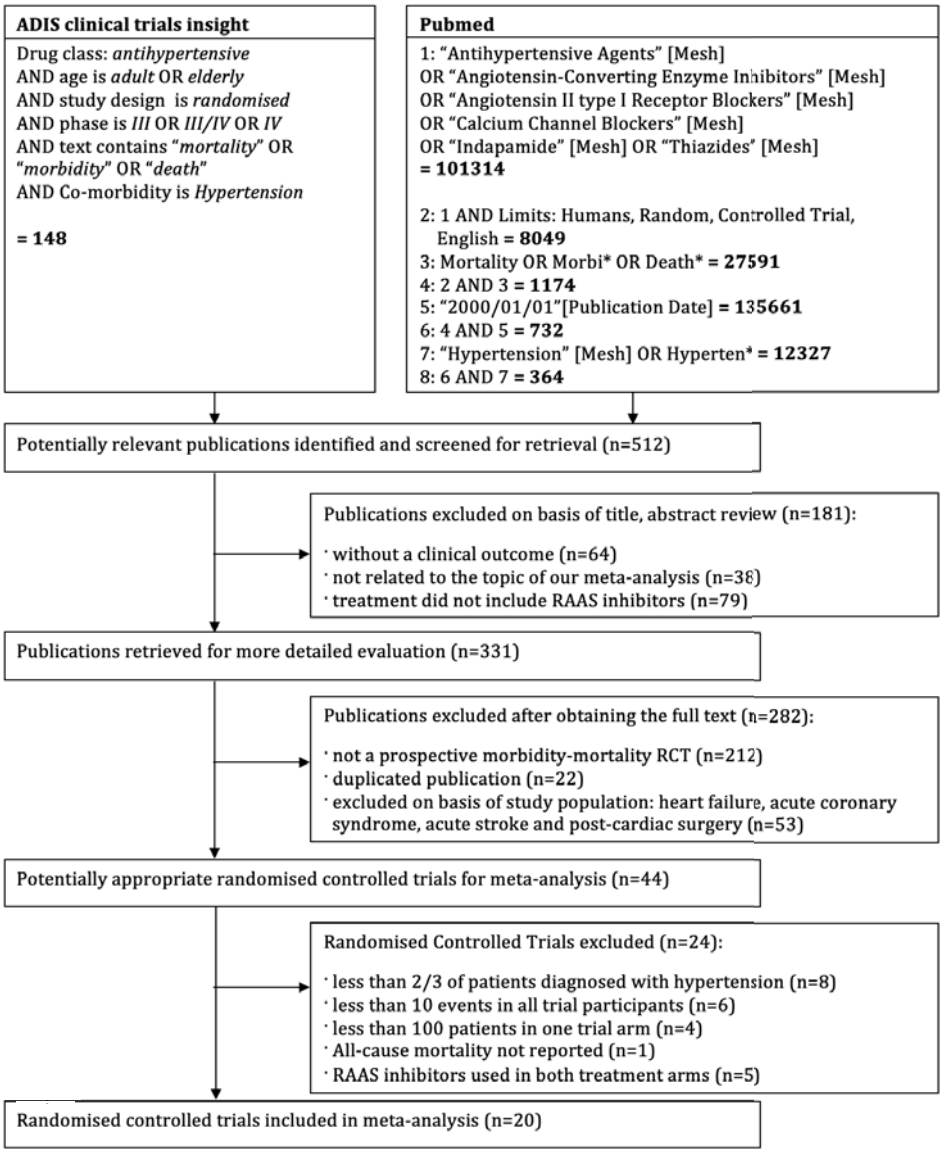

Figure 1. Flow diagram of trial search and selection process.
RAAS= renin-angiotensin-aldosterone system, RCT=randomized clinical trials.

Endpoint definition

The endpoints of this pooled analysis were all-cause and cardiovascular mortality during long-term follow-up. Data on all-cause death were available for all trials. Data on

cardiovascular death were not available for RENAAL, IDNT, MOSES, and CASE-J.

We aimed to provide estimates of the incidence of these endpoints in patients randomized to RAAS inhibitors and control therapy, as well as estimates of the absolute and relative reduction in the incidence of the endpoints by RAAS inhibitors. Since the duration of follow-up varied between the trials, we decided to base our analyses on the mortality incidence rate (IR), which was assumed to be constant over time in each of the comparison groups. The IR is defined as the number of patients who reached the endpoint in the comparison group divided by the patient-years of follow-up in the corresponding group (i.e. the sum of the follow-up times for each individual). The latter figure is equal to the number of patients multiplied by their mean follow-up duration.

To obtain the trial- and treatment-arm specific mean follow-up duration, the following five-step approach was applied. Firstly, we observed whether the mean follow-up time per treatment arm was stated in the paper. If this was not available, we then derived it from the reported death rate by dividing the total number of deaths by the annual death rate. If these data were not available, then the mean follow-up time was estimated from incidences that were derived from Kaplan–Meier curves, in combination with the number of patients that were reported to be at risk at several follow-up points. Finally, if we were not able to compute the mean follow-up duration for each treatment arm separately, we used the mean follow-up time that was reported for all trial participants together.

Statistical analysis

For each individual trial, the treatment-arm specific all-cause and cardiovascular mortality IR was determined. We evaluated the assumption that the mortality rate is constant over time by visually inspecting the Kaplan–Meier curves of the studies in this meta-analysis, comparing different time windows within each Kaplan–Meier curve. We did not find any major deviation from this assumption. Furthermore, we realized that the follow-up time within each of the trials is relatively short (the overall mean follow-up duration is 4.3 years). Thus, on average, during the course of the trial, patients became only 4 years older. In view of this fact, it seems reasonable to assume that the IRs were constant over time.

Information on follow-up times is needed to obtain estimates of absolute risks (and absolute treatment effects). However, because of the assumptions that we used, our IR estimates might be somewhat inaccurate. Therefore, we based our estimates of relative treatment effects on the hazard ratios (HRs) and confidence intervals (CIs) or standard errors that were reported for each trial. Actually, HRs were available for all trials, except

for RENAAL, SCOPE, and pilot HYVET. For these trials, we calculated HRs based on the IRs in the separate treatment arms.

Because of the large variety in active (and control) treatments, we used a random-effects model to compute an overall pooled HR, even in case statistical tests for heterogeneity across trials were non-significant. Statistical heterogeneity was tested by Cochran's Q statistic,[17] and a *P*-value <0.10 (two sided) was considered to indicate heterogeneity among trials. The degree of heterogeneity was presented as an I^2 value. Publication bias was assessed by visually examining funnel plot asymmetry and quantified by using an Egger regression test to calculate two-tailed *P*-values.[18]

We hypothesized that the mortality reduction by antihypertensive drugs might be influenced by age, gender, baseline SBP, BP reduction during follow-up, and follow-up time. To evaluate this hypothesis, we conducted linear regression analyses, based on trial-level data (so-called 'meta-regression'). The trial-specific mean age, percentage of men, mean SBP, mean difference in BP reduction after 1 year of follow-up between RAAS inhibitors and control therapy, and mean follow-up time were considered as explanatory variables of the natural logarithm of the trial-specific hazard ratio (lnHR) for all-cause mortality. In this analysis, trial-level observations were weighed according to the inverse of the squared standard error of lnHR, thus taking into account the amount of 'statistical information' that is produced by each trial. Secondly, by including follow-up time in this analysis we were able to see if the mortality incidence ratio is constant over time.

Although we hypothesized that, taken all evidence together, RAAS inhibitors as a class of drugs would produce a homogenous treatment effect in terms of a mortality reduction compared with (contemporary) control therapy, we also performed stratified analyses according to the class of drug (ACE inhibitor vs. ARBs), as we realized that ACE inhibitors and ARBs have partly different modes of action. We also performed stratified analyses according to the type of control (placebo vs. active treatment), and percentage of patients with diabetes mellitus or renal insufficiency at baseline (>50% vs. <50%). Pooled HRs for all-cause mortality were determined using a random effects model for each stratum, and differences between strata were studied.

All statistical tests were two-sided, and a *P*-value <0.05 was considered significant. We used SAS 9.2 for Windows for data analysis.

Results

Trial characteristics

A total of 20 trials fulfilled all selection criteria for this meta-analysis, and their main characteristics are presented in Table 1.[9-11,19-35] In total 158,998 patients were randomized to RAAS inhibitor therapy (n= 71 401; 299 982 patient-years of follow-up) or control treatment (n= 87 597; 377 023 patient-years of follow-up). ACE inhibitors were used as the active treatment in seven trials (n= 76 615); two of these studies were placebo-controlled.[23,24,26,30,31,33,34] Thirteen trials, of which five were placebo-controlled, allocated participants to an ARB as the active treatment (n= 82 383).[9-11,19-22,25,27-29,32,35]

Patient characteristics

On average, 91% of the trial participants were hypertensive according to the definition used in each trial. The mean baseline SBP was 153 mmHg (range of the means across trials 135-182), the mean age was 67 years (range of the means across trials 59-84) and 58% of participants were man (range of this percentage across trials 36-80; Table 1).

All-cause mortality

During a mean follow-up of 4.3 years, 6284 of the patients assigned to an RAAS inhibitor reached the endpoint of all-cause death. This corresponds with an IR of 20.9 deaths per 1000 patient-years. During the same period, a total of 8777 patients assigned to control therapy had all-cause death, implying an IR of 23.3 deaths per 1000 patient-years. RAAS inhibition was associated with a statistically significant reduction in all-cause mortality in three individual trials, ASCOT-BPLA, ADVANCE and HYVET (Figure 2).[23,26,31]

In all 20 trials grouped together, treatment with a RAAS inhibitor was associated with a statistically significant 5% reduction in all-cause mortality (HR 0.95, 95% CI 0.91 to 1.00, *P*= 0.032; Figure 2). The degree of heterogeneity in treatment effect across all trials was low (I^2 15%) and non-significant (*P*= 0.266). No funnel-plot asymmetry was visualized, and the *P*-value using an Egger regression test for all-cause mortality was >0.10 (intercept -0.3, 95% CI -1.3-0.68; *P*= 0.53), indicating no evidence for publication bias.

Cardiovascular mortality

Excluding the four trials that did not report on cardiovascular mortality, 2570 patients assigned to RAAS inhibition had cardiovascular death. Based on a total of 295 617 patient-years of follow-up, the IR was 8.7 per 1000 patient-years. The IR in patients assigned to control therapy was 10.1 per 1000 patient-years (3773 events; 372 105 patient-years of follow-up), resulting in a significant 7% overall reduction in cardiovascular mortality (HR:

Table 1. Baseline characteristics f study population in 20 trials (n=158 998).

Trial acronym	Year	N	Active drug	Control	Mean follow-up, years	Hyper-tension, %	Mean SBP, mmHg	Mean age, (years)	Men, %	IR in control group
RENAAL[9]	2001	1513	Losartan	Placebo	3.09	96.5	153	60.0	63.2	66.0
IDNT[28]	2001	1715	Irbesartan	Amlodipine or placebo	2.86	100	159	58.9	66.5	54.0
LIFE[25]	2002	9193	Losartan +/- HCTZ	Atenolol +/- HCTZ	4.82	100	174	66.9	46.0	19.5
ALLHAT[30]	2002	33357	Lisinopril	Chlorthalidone or amlodipine	5.01	100	146	66.9	53.3	28.5
ANBP-2[33]	2003	6083	ACE inhibitor (enalapril)	Diuretic (HCTZ)	4.06	100	168	71.9	49.0	17.1
SCOPE[29]	2003	4937	Candesartan	Placebo	3.74	100	166	76.4	35.5	29.0
pilot HYVET[24]	2003	1283	Lisinopril	Diuretic or no treatment	1.12	100	182	83.8	36.6	55.4
JMIC-B[34]	2004	1650	ACE inhibitor	Nifedipine	2.25	100	146	64.5	68.8	6.2
VALUE[27]	2004	15245	Valsartan	Amlodipine	4.32	100	155	67.3	57.6	24.8
MOSES[32]	2005	1352	Eprosartan	Nitrendipine	2.50	100	152	68.1	54.2	31.0
ASCOT-BPLA[26]	2005	19257	Amlodipine +/- perindopril	Atenolol +/- bendroflumethiazide	5.50	100	164	63.0	76.6	15.5
JIKEI HEART[11]	2007	3081	Valsartan	Non-ARB	2.81	87.6	139	65.0	66.3	6.2
ADVANCE[31]	2007	11140	Perindopril + indapamide	Placebo	4.30	68.7	145	66.0	57.5	19.8
HYVET[23]	2008	3845	Indapamide +/- perindopril	Placebo	2.11	89.9	173	83.6	39.5	59.3
PRoFESS[22]	2008	20332	Telmisartan	Placebo	2.50	74.0	144	66.2	64.0	29.1
TRANSCEND[35]	2008	5926	Telmisartan	Placebo	4.67	76.4	141	66.9	57.0	25.2
CASE-J[20]	2008	4703	Candesartan	Amlodipine	3.30	100	163	63.8	55.2	11.1
HIJ-CREATE[19]	2009	2049	Candesartan	Non-ARB	4.03	100	135	64.8	80.2	14.3
KYOTO HEART[21]	2009	3031	Valsartan	Non-ARB	2.92	100	157	66.0	57.0	7.2
NAVIGATOR[10]	2010	9306	Valsartan	Placebo	6.10	77.5	140	63.8	49.4	11.5

HCTZ = Hydrochlorothiazide, ACE = Angiotensin-Converting Enzyme, ARB = Angiotensin-Receptor Blocker, SBP=Systolic Blood Pressure, IR=Incidence Rate per 1000 patient years.

0.93, 95% CI: 0.88–0.99, *P*= 0.018; Figure 2). The degree of heterogeneity in treatment effect across all trials was low (I^2: 23%) and non-significant (*P*= 0.194). There was no evidence of publication bias.

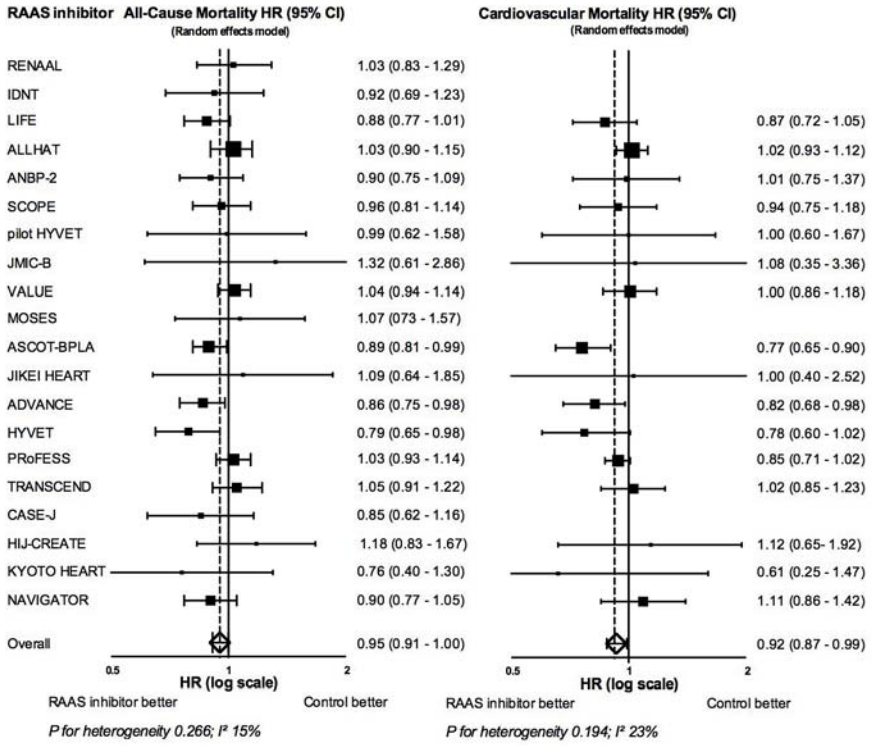

Figure 2. All-cause and cardiovascular mortality treatment effect of renin–angiotensin–aldosterone system blockade in all included trials.
HR=Hazard Ratio, CI=Confidence Interval, RAAS= renin-angiotensin-aldosterone system.
Overall p-value 0·032 for all-cause mortality. Overall p-value 0.018 for cardiovascular mortality.

Angiotensin-converting enzyme inhibitors vs. AT1 receptor blockers

All seven trials together, ACE inhibitors were associated with a statistically significant 10% reduction in all-cause mortality (IR: 20.4 vs. 24.2 deaths per 1000 patient-years; HR: 0.90, 95% CI: 0.84–0.97, *P*= 0.004). No significant mortality reduction could be demonstrated with ARB treatment (13 trials; IR: 21.4 vs. 22.0 deaths per 1000 patient-years; HR: 0.99, 95% CI: 0.94–1.04, *P*= 0.683). This difference in the treatment effect between ACE inhibitors and ARBs was statistically significant (*P*-value for interaction 0.036). Apparently, the observed mortality reduction in the overall group of RAAS

inhibitors was completely driven by the beneficial effect of the ACE inhibitors.

As far as the ACE inhibitor trials are concerned, the largest mortality reductions were observed in ASCOT-BPLA, ADVANCE, and HYVET, all of which studied the ACE inhibitor perindopril (pooled HR: 0.87, 95% CI: 0.81–0.93, *P*-value <0.001). However, there was no evidence of heterogeneity among the ACE inhibitor trials in the effect of the studied ACE inhibitor regimen on all-cause mortality (*P*-value for heterogeneity 0.310, I^2: 16%; Figure 3). There was also no evidence of heterogeneity in the effect of ARBs (*P*-value for heterogeneity 0.631, I^2: 0%).

Patients randomized to an ACE inhibitor had 9.1 cardiovascular deaths per 1000 patient-years, compared with 11.2 in their controls (HR: 0.88; 95% CI: 0.77–1.00; *P*= 0.051). In the ARB trials, the IRs were 8.8 and 9.2 cardiovascular deaths per 1000 patient-years for patients assigned to ARB and control therapy, respectively (HR: 0.96; 95% CI: 0.90–1.01; *P*= 0.143). The test for heterogeneity in effects on cardiovascular mortality between ACE inhibitors and ARBs was statistically non-significant (*P*= 0.227).

Meta-regression

Multiple linear regression analysis showed a significant (*P*= 0.035) association between the trial-specific mean SBP (measured at baseline), and the relative mortality reduction by RAAS-blockade. The mortality reduction was largest in trials with the highest mean baseline BP values. Secondly, there was a significant (*P*= 0.008) relation between the trial specific mean difference in BP between RAAS inhibitors and control therapy at 1-year follow-up, and the mortality reduction produced by RAAS inhibitors. The mortality reduction was largest in trials with the largest difference in mean SBP reduction. No significant association was found between the trial-specific mean age, man/woman ratio, mean follow-up time and the mortality reduction by RAAS-blockade. Mean follow-up time was also not related to the observed mortality reduction, supporting our hypothesis that the mortality incidence ratio is constant over time (at least for the mean duration of 4.3 years).

Stratified analyses

Similar HRs for all-cause mortality were found in clinical trials that compared RAAS inhibition with placebo (HR 0.95, 95% CI 0.88 to 1.02, *P*= 0.177) and with active control (HR 0.95, 95% CI 0.91 to 1.01, *P*= 0.066; *P*-value for interaction 0.889). Likewise, no heterogeneity in treatment effect was observed with respect to the percentage of participants with diabetes mellitus or renal insufficiency.

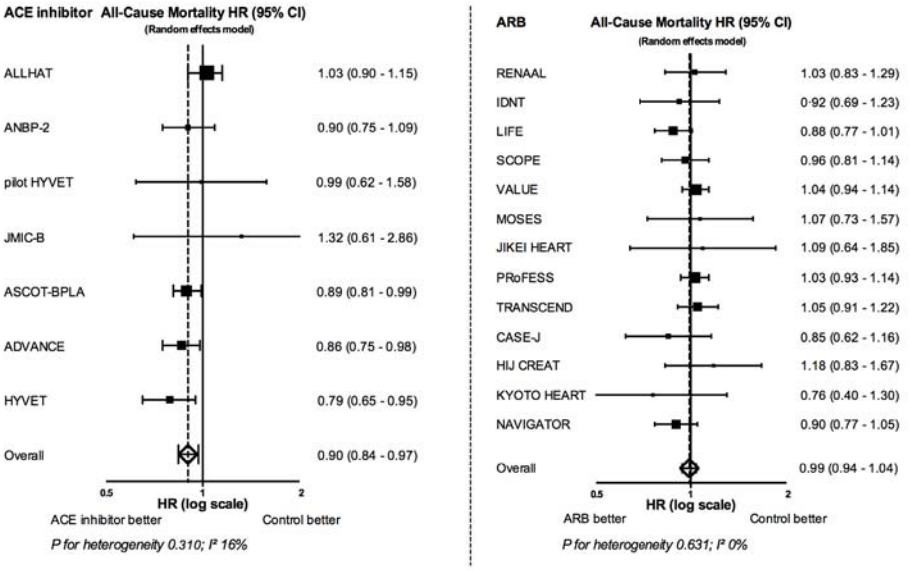

Figure 3. All-cause mortality treatment effect of ACE-inhibitor and ARB trials.
HR=Hazard Ratio, CI=Confidence Interval, ACE=Angiotensin-Converting Enzyme, ARB=Angiotensin Receptor Blocker. P= 0.004 for the treatment effect of ACE inhibitor on all-cause mortality. P= 0.683 for the treatment effect of ARB on all-cause mortality.

Discussion

This meta-analysis, which included almost 160 000 patients, sought to evaluate the effect of RAAS inhibitors as a class of drugs on total and cardiovascular mortality in their main indication hypertension. Overall, the results show a 5% reduction in all-cause mortality during a 4-year follow-up period associated with the class of RAAS inhibitors. This mortality reduction was found when compared with placebo, as well as in comparison with other BP-lowering drugs. However, in a stratified analysis according to the class of drug, it was shown that the observed overall all-cause mortality reduction was almost completely a result of the beneficial effect of the class of ACE inhibitors (10% relative reduction in all-cause mortality), whereas the ARBs showed a neutral treatment effect. The findings are firm, as the analysis included a large number of patient-years (677 005) and endpoints (15 061 deaths). The findings are relevant to clinical practice, as they are based on data from well-designed randomized trials encompassing a broad population of patients with high BP, who were well-treated for concomitant risk factors and who represent usual hypertensive patients seen today.

Reduction in mortality is the primary goal of antihypertensive therapy.[2] Paradoxically, the effect of RAAS inhibitors on mortality in hypertensive patients remained uncertain and had never been systematically evaluated. To our knowledge, no prior published meta-analysis investigated the efficacy of RAAS inhibitors on all-cause and cardiovascular mortality in their main indication of hypertension. Previous analyses in for example heart failure or coronary artery disease populations (with or without hypertension) demonstrated a reduction in cardiovascular events, stroke, and mortality.[36,37] In addition, a pooled analysis of trials in patients with cardiovascular disease (including hypertension) concluded that the reduction in cardiovascular mortality and stroke with RAAS inhibitors is BP dependent.[38] In our analyses, the significant reduction in cardiovascular mortality associated with RAAS inhibition supports previous literature.

As stated, the primary aim of this meta-analysis decided a priori was to test the hypothesis that RAAS inhibitors as a class of drugs would have a beneficial effect on total mortality in hypertension, when compared with contemporary control antihypertensive therapy. However, as we realized that, among the RAAS inhibitors, the ACE inhibitors and ARBs have different mechanisms of action, we also decided to study whether there was a differential effect on mortality between these two classes of drugs. Indeed, our analysis clearly showed that nearly all of the mortality reduction was observed with ACE inhibitors. Contrary, there was no clear benefit from the ARBs. This was supported by the sensitivity analysis, which showed a significant stronger treatment effect in the ACE inhibitor trials compared with the ARB trials. With respect to this finding several points deserve consideration.

The reduced effect of ARBs on mortality when compared with ACE inhibitors has also previously been discussed.[39, 40] A recent meta-analysis of 37 ARB trials also failed to detect a reduction in all-cause or cardiovascular mortality in a broad population of patients.[41] The differences in the modes of action between ACE inhibitors and ARBs, and the small-but-definite BP-independent reduction in CAD mortality with ACE inhibitors, which has not been observed with ARBs or other antihypertensive agents, might contribute to this finding.[42] On the other hand, others have demonstrated that BP-dependent beneficial effects in the prevention of stroke and heart failure are similar for ACE inhibitors and ARBs. ACE inhibitors and ARBs have also been shown to be equally effective in preventing atrial fibrillation and new-onset diabetes.[43,44] Furthermore, it should be emphasized that we did not design this meta-analysis to make a head-to-head comparison between ACE inhibitors and ARBs. The finding that the beneficial effect is seen in the ACE inhibitor population as opposed to the ARB population should be considered a post hoc observation. Given the nature of meta-analyses, which are per definition data-driven,

the differential effect between ACE inhibitors and ARBs should be interpreted with caution to avoid overstating this subgroup finding vis-à-vis the a priori hypothesis. In this respect it should also be noted that the difference in effect on cardiovascular mortality between ACE inhibitors and ARBs was not statistically significant. Furthermore, two previous studies were designed to compare ACE inhibitors and ARBs in an hypertensive population, but both the ONTARGET (telmisartan vs. ramipril) and DETAIL (telmisartan vs. enalapril) trial did not show a differential treatment effect between ARBs and ACE inhibitors.[15,45] Thus, at present, the results of this analysis do not warrant changing clinical practice treatment guidelines that recommend that an ARB may be used in ACE inhibitor-intolerant hypertensive patients.[2] Hopefully, our findings will form the basis of further analysis and studies into the effects of BP treatment and total mortality, which is the first line priority in the guidelines for the management of hypertension.

It might be argued that the observed 5% relative mortality reduction in the overall group of RAAS inhibitors, and the 10% relative mortality reduction in the ACE inhibitor group is small, and only found to be statistically significant in our analysis because of statistical 'overpowering'. Indeed, in meta-analyses clinically irrelevant treatment effects might become statistically significant (i.e. the estimated effect divided by the standard error is >1.96) simply because of the large size of the aggregate (or pooled) trials. In our view, however, the observed mortality reduction in this meta-analysis is clinically relevant indeed, for several reasons. Firstly, it should be realized that the treatment effect was reached in patients who did receive a broad range of other contemporary risk-reduction therapies, including statins, antiplatelet therapy, beta-blockers, diuretics, and other BP-lowering medication (note that, as per design, we included trials that were conducted during 2000–2011). Secondly, the estimated absolute mortality reduction was 2.4 per 1000 patient-years for the RAAS inhibitors as a group and 3.8 per 1000 patient-years for the class of ACE inhibitors. This is an interesting figure, particularly since the prevalence of hypertension in Western (CAD) populations is high,[46] despite the widespread use of BP-lowering medication. Thus a wider application of these agents, in particular of ACE inhibitors, may have substantial effects on the population level. Interestingly, the observed mortality reduction was largest in trials with the highest baseline SBP. The observed mortality reduction may be used as an additional argument to stimulate patients to adhere to the prescribed treatment.

Limitations

Several limitations of our analysis have to be mentioned. Firstly, there was a great deal of variation between the studied populations. For example, trials used different definitions of hypertension, different dosages of the active and control drug, different target BP

levels, different follow-up times, and in several studies patients had other concomitant conditions and background therapy. Although this does not hamper the generalizability of our results, it makes it challenging to accurately estimate the effect of RAAS inhibition in a broad range of routine clinical practice situations.

Secondly, this meta-analysis is based on trial level data, rather than on individual patient data. Information on background therapy and co-morbidities were not available in several trial reports. Thus, we could not reliably analyse the relation between these factors and the observed mortality reduction. Moreover, the treatment arm-specific follow-up time was not available in all trials, we therefore derived follow-up time from either the reported death rate, Kaplan–Meier curves, or mean follow-up duration. This is an approximation of the true follow-up time, and we appreciate that our estimates of mortality incidence might be somewhat over or underestimated. However, importantly, this methodology had not influenced the estimation of the observed relative mortality reduction, which was mainly based on the HRs that were reported for the separate trials.

Finally, this meta-analysis assumed a class effect among the different ACE inhibitors and ARBs. The validity of this concept was not challenged by formal statistical tests on heterogeneity of treatment effects among the different (ACE inhibitor and ARB) trials. Still, it should be realized that differences may exist between drugs within the same class that are simply missed due to lack of statistical power. It should therefore be emphasized that our findings should be interpreted in relation to the pharmacological properties of the applied agents.

Conclusion

This meta-analysis, which involved almost 160 000 patients, demonstrated that RAAS inhibitors as a class of antihypertensive drugs were associated with a significant 5% relative reduction in all-cause mortality in populations with a high prevalence of hypertension when compared with contemporary control antihypertensive therapy. Stratified subgroup analysis according to class of drug showed a differential treatment effect between ACE inhibitors and ARBs. The overall reduction in all-cause mortality resulted almost completely from the class of ACE inhibitors, which were associated with a statistically significant 10% relative reduction in all-cause mortality, whereas no mortality reduction was observed with the ARBs. In view of the high prevalence of hypertension in the general population, widespread use of ACE inhibitors may therefore

result in a considerable gain in lives saved. The results of this study provide a convincing argument to improve treatment adherence in the millions of people around the world suffering from hypertension and its sequelae.

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Conflict of interest

M.B reports to have previously received research grants, fees, and honoraria from: Merck-Sharpe Dohme, American Medicine Company, Lilly, Servier, and Sanofi-Aventis. K.F. receives fees, and research grants from Servier Laboratories. During the previous 5 years, J.J.M. has received fees for an occasional consultancy or guest speaker meeting from various pharmaceutical companies. L.C.v.V., K.M.A., J.J.B., and E.B. have no conflict of interest to report.

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Meta-analysis of RCTs of RAAS inhibitors after the retraction of the kyoto

17 March 2013 Eric Boersma
Head Clinical Epidemiology Unit, Erasmus Medical Center
Rotterdam, March 7, 2013

The Editorial Board of your Journal has decided to retract the paper reporting the main results of the Kyoto Heart Study (Eur Heart J 2009;30:2461-2469). We studied the impact of this decision on the findings and conclusion of our meta-analysis of randomized clinical trials of renin-angiotensinaldosterone system (RAAS) inhibitors, published in the European Heart Journal (Eur Heart J 2012;33:2088-97), which included the Kyoto Heart Study.

In summary, excluding the Kyoto Heart Study, our meta-analysis involves 19 clinical trials, including 155967 patients (69884 RAAS inhibitor; 86083 control). The incidence of all-cause death was 21.2 and 23.5 per 1000 patient-years in patients randomized to RAAS inhibition and controls, respectively. Overall, RAAS inhibition was associated with a 5% reduction in all-cause mortality (HR: 0.95, 95% CI: 0.91-1.00, P = 0.039), and a 7% reduction in cardiovascular mortality (HR: 0.93, 95% CI: 0.88-0.99, P = 0.022). The observed treatment effect resulted entirely from the class of ACE inhibitors, which were associated with a significant 10% reduction in all-cause mortality (HR: 0.90, 95% CI: 0.84-0.97, P = 0.004), whereas no mortality reduction could be demonstrated with ARB treatment (HR: 0.99, 95% CI: 0.95-1.04, P = 0.729). This difference in treatment effect between ACE inhibitors and ARBs on all-cause mortality was statistically significant (P for heterogeneity = 0.033).

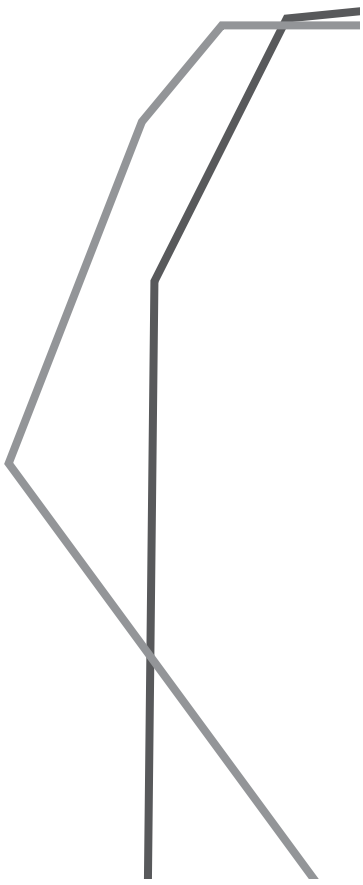
Thus, after excluding the Kyoto Heart Study, the conclusion of our meta-analysis remains unchanged: in patients with hypertension, treatment with an ACE inhibitor results in a significant further reduction in all-cause mortality. Because of the high prevalence of hypertension, the widespread use of ACE inhibitors may result in an important gain in lives saved.

Sincerely,

Eric Boersma, on behalf of the co-authors Erasmus MC Rotterdam The Netherlands

Conflict of Interest

None declared



Chapter 12

Impact of renin-angiotensin system inhibitors on mortality and major cardiovascular endpoints in hypertension: A number-needed-to-treat analysis

J.J. Brugts, L.C. van Vark, K.M. Akkerhuis, M. Bertrand, K. Fox, J.J. Mourad, E. Boersma

Int J Cardiol. 2015 Feb 15

Abstract

Objective

To assess the effectiveness of renin–angiotensin aldosterone system (RAAS) inhibitors to prevent all-cause and cardiovascular death, myocardial infarction and stroke in hypertensive patients considering the number needed to treat (NNT).

Methods

Data from a meta-analysis of 18 prospective, randomized, controlled morbidity–mortality trials (68 343 RAAS inhibitor; 84 543 control) were used to calculate NNTs for the prevention of all-cause and cardiovascular mortality, myocardial infarction, and stroke.

Results

Angiotensin-converting enzyme (ACE) inhibitors were used in 7 trials and angiotensin receptor blockers (ARBs) in 11 trials. Mean follow-up was 4.3 years. The annual incidence rate of all-cause mortality was 0.0233 in patients randomized to RAAS inhibitors versus 0.0252 in controls (hazard ratio 0.95, 95% confidence interval 0.91 to 0.99). The corresponding median NNT to prevent one death was 113 (2.5–97.5th percentile, 85 to 168) in favor of RAAS inhibitors, which was driven by ACE inhibitors (NNT 67, 2.5–97.5th percentile, 53 to 92) rather than ARBs (NNT 335, 2.5–97.5th percentile, − 4341 to 5076). Results for cardiovascular mortality (NNT 116 for ACE inhibitors and 409 for ARBs, respectively) and myocardial infarction (NNT 80 and 338, respectively) also appeared to be driven by ACE inhibitors. We found a lower NNT for stroke in favor of ARB (NNT 337 and 131, respectively) although this difference was statistically non-significant.

Conclusion

Among hypertensive patients, ACE inhibitors but not ARBs, substantially reduce all-cause and cardiovascular mortality and myocardial infarction.

Introduction

With 13% of deaths worldwide being attributable to hypertension-related diseases, hypertension is considered by the World Health Organization as the number one cause of mortality.[1] This observation is consistent with current understanding of antihypertensive therapies, which reduce blood pressure (BP) and cardiovascular morbidity, but do not necessarily improve mortality. Renin–angiotensin aldosterone system (RAAS) inhibitors, for example, have an array of effects on morbidity in hypertensive patients, including prevention of the onset of diabetes, nephroprotection, and vascular protection.[2-5] However, the effect on mortality in hypertension has only recently been evaluated in a meta-analysis by our group.[6]

Within the class of RAAS inhibitors, both the angiotensin-converting enzyme (ACE) inhibitors and the angiotensin receptors blockers (ARBs) target the RAAS. However, their mechanisms of action are quite different,[7] which may obviously influence their clinical effectiveness. Indeed, in our recent meta-analysis of randomized controlled RAAS inhibitor trials in hypertension, the hazard ratio for all-cause mortality was 0.90 (0.84–0.97) for ACE inhibitors and 0.99 (0.94–1.04) for ARBs during 4.3 years of follow-up. We concluded that ACE inhibitors – but not ARBs – reduce all-cause mortality in hypertensive patients.[6] These results have subsequently been confirmed in similar meta-analyses in different patient populations.[8-11] Interestingly, current guidelines for the treatment of hypertension do not distinguish between these two types of RAAS inhibitors. The current efficacy paradox between ACE inhibitors and ARBs for reducing mortality and CV endpoints is a topic of intensive debate worldwide for which additional analyses are essential. Information on absolute treatment effect, summarized as 'number needed to treat' is essential for future guidelines and health care policies.

Our meta-analytical data were presented in terms of relative risk reductions on all-cause mortality. Presenting a global picture of the impact of any treatment on risk, with the inclusion of absolute risk reductions, and the number of patients needed to treat (NNT) to prevent one additional adverse outcome is novel and highly important.[12] In one example unrelated to hypertension, third-generation oral contraceptive pills were reported to be associated with a two-fold increase in thrombosis (i.e. relative risk), while closer examination of the data indicated that this corresponded to an increase from 1 event per 7000 women to 2 events per 7000 women (i.e. absolute risk). In that particular case, misinterpretation of the data had a negative impact on public health in terms of unwanted pregnancies and abortions.[12] Together with other examples,[13] this underlines the importance of considering all parameters associated with the impact of treatment

on risk. In this context, the European Medicines Agency has also endorsed the NNT.[14]

In this article, we assessed the effectiveness of renin–angiotensin aldosterone system (RAAS) inhibitors to prevent all-cause and cardiovascular death, myocardial infarction and stroke in hypertensive patients. We present separate NNTs for ACE inhibitors and ARBs on these endpoints. In view of the high prevalence of hypertension and its impact on worldwide mortality and morbidity, implications of these findings are substantial.

Methods

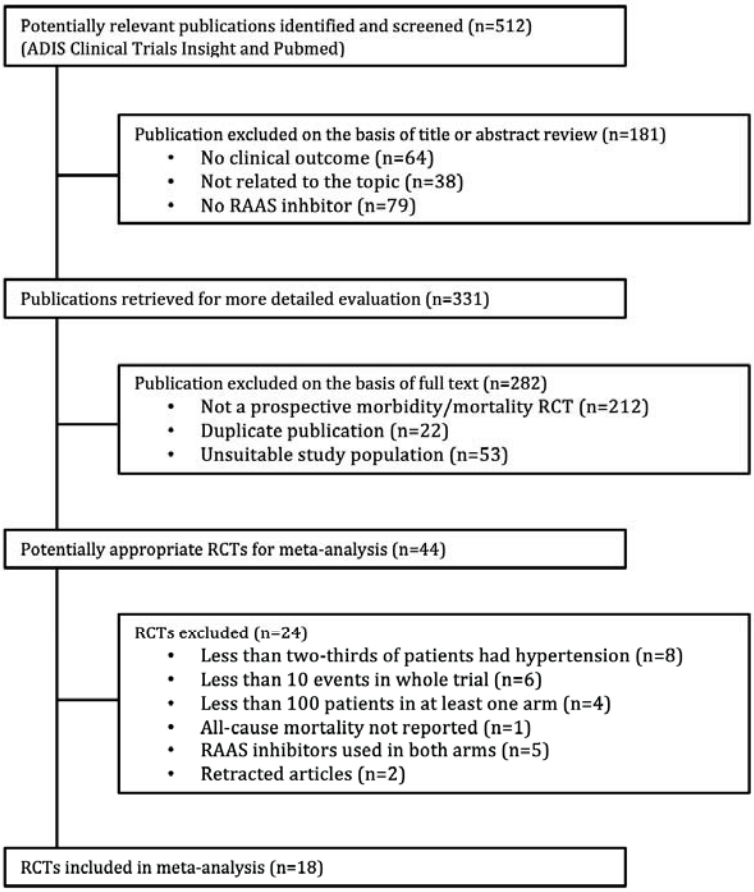
Trial selection

Data from a pooled meta-analysis of 18 trials described in the Supplementary Webtable 1,[15] which included 152,886 patients (68,343 RAAS inhibitor; 84,543 control), were used to calculate NNTs. Detailed methods for identifying trials and extracting data have been described elsewhere, and a QUOROM diagram is shown in Webfigure 1.[6,15] In summary, prospective, randomized, controlled morbidity–mortality trials in which one arm included treatment with a RAAS inhibitor (ACE inhibitor or ARB) were included if they were published between January 1, 2000 and March 1, 2011, if all-cause mortality was reported in the principal study publication, if all-cause mortality occurred in at least 10 patients, if the RAAS treatment group included at least 100 patients in one trial arm, if the comparator group was treated with placebo or an antihypertensive treatment regimen other than a RAAS inhibitor, if at least two-thirds of patients were hypertensive according to the definition used in each trial, and if follow-up time was at least 1 year. Trials in which patients had been selected because of a specific disease such as heart failure, acute coronary syndrome, acute stroke, hemodialysis, atrial fibrillation, or post-cardiac surgery were excluded, in order to select a meta-analytical population with a homogeneous risk profile. Compared with our previous reports, the analysis described here excluded two trials due to retractions of the original articles describing the results (the KYOTO heart study and the JIKEI trial) (Webfigure 1).[6,15]

Endpoints

We studied the effect of RAAS inhibitors on five endpoints: all-cause mortality, cardiovascular mortality, myocardial infarction, stroke, and the composite of myocardial infarction or stroke. The trial-specific definitions of the endpoints were maintained. All the trials reported all-cause mortality. For the other events, only trials reporting the endpoint concerned were included in the corresponding analysis, i.e. 15 trials for cardiovascular mortality (64,557 patients RAAS inhibitor, 80,761 patients control), 17

trials for myocardial infarction (67,912 patients RAAS inhibitor, 83,691 patients control), 17 trials for stroke (67,592 patients RAAS inhibitor, 83,781 patients control), and 16 trials for the composite of myocardial infarction or stroke (67,161 patients RAAS inhibitor, 82,929 patients control).



Webfigure 1. Flow diagram for search of trials and selection process (QUOROM). RCT, randomised controlled trial. RAAS, renin-angiotensin-aldosterone inhibitor. Reproduced in part from reference 6.

Statistical analysis

Trial-specific, treatment-arm-specific and endpoint-specific incidence rates (IR) were determined as the ratio of the number of endpoint events and the patient years of follow-up. The follow-up time within each of the trials was relatively short (the overall

mean follow-up duration is 4.3 years). Consequently, on average, during the course of the trial, patients became only 4 years older. Therefore, we assumed that the IRs were constant over time (we did not find any major deviation from this assumption by visually inspecting the Kaplan–Meier curves of the studies in this meta-analysis), and hazard ratios (HR) were then determined as the ratio of the IR in the patients randomized to RAAS inhibitors, divided by the IR in controls. We report HRs together with their 95% confidence interval (CI). We used a logarithmic scale in Fig. 1 to express NNTs because of the non-normal distribution of NNTs. Pooled estimates were obtained by weighing trial-level observations according to the inverse of the squared standard error of the natural logarithm of the HR, thus taking into account the amount of "statistical information" that is produced by each trial.

Based on the obtained IRs, trial-specific (and pooled) NNT values were calculated as the number of patients that need to be treated in order to avoid one event over 4.3 years, using the inverse of the absolute risk reductions, and taking into account the corresponding follow-up duration. Simulation analyses (10,000 for each NNT) were performed to obtain point estimates and estimates of precision. We report the median and the 2.5th to 97.5th percentile of the simulated distribution of NNTs. More precisely, we sampled 10,000 times from normal distributions (RAAS inhibitors and control) with μ = the natural logarithm of the observed IR (lnIR), and σ = standard error of lnIR. The obtained values were then raised to the power of e, subtracted and inversed.

All analyses are performed for RAAS inhibitors as a class, and for ACE inhibitors and ARBs separately.

Results

ACE inhibitors were used as the active treatment in 7 trials and ARBs in 11 trials (**Webtable 1**). Follow-up in the trials ranged from 1432 (pilot HYVET) to 167,099 (ALLHAT) patient-years, with a mean follow-up duration of 4.3 years. The baseline characteristics of the population were similar to those presented elsewhere.[6]

Webtable 1. Trials included in the meta-analysis.

Trial	Type of RAAS inhibitor	RAAS inhibitor		Control	
		Number of patients	Follow-up, patient-years	Number of patients	Follow-up, patient-years
RENAAL	ARB	751	2324	762	2349
IDNT	ARB	579	1652	1136	3260
LIFE	ARB	4605	22 190	4588	22 076
ALLHAT	ACE inhibitor	9054	45 837	24 303	12 1262
ANBP-2	ACE inhibitor	3044	12 420	3039	12 281
SCOPE	ARB	2477	9283	2460	9172
pilot HYVET	ACE inhibitor	431	493	852	939
JMIC-B	ACE inhibitor	822	1784	828	1931
VALUE	ARB	7649	32 852	7596	32 984
MOSES	ARB	681	1703	671	1678
ASCOT-BPLA	ACE inhibitor	9639	53 094	9618	52 903
ADVANCE	ACE inhibitor	5569	24 005	5571	23 845
HYVET	ACE inhibitor	1933	4159	1912	3964
PRoFESS	ARB	10 146	25 365	10 186	25 465
TRANSCEND	ARB	2954	13 785	2972	13 869
CASE-J	ARB	2354	7766	2349	7748
HJ-CREATE	ARB	1024	4132	1025	4126
NAVIGATOR	ARB	4631	28 365	4675	28 435
Total		**68 343**	**291 208**	**84 543**	**368 285**

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker.
Details of trials: ADVANCE 2007: perindopril/indapamide vs placebo; ALLHAT 2002: lisinopril vs chlorthalidone or amlodipine; ANBP-2 2003: enalapril or other ACE inhibitor vs hydrochlorothiazide or other diuretic; ASCOT-BPLA 2005: amlodipine ± perindopril vs atenolol ± bendroflumethiazide; CASE-J 2008: candesartan vs amlodipine; HIJ-CREATE 2009: candesartan vs non-ARB; HYVET 2008: indapamide ± perindopril vs placebo; Pilot HYVET 2003: lisinopril or other ACE inhibitor vs bendroflumethiazide (or other diuretic) or no treatment; IDNT 2001: irbesartan vs amlodipine or placebo; JMIC-B 2004: ACE inhibitor vs nifedipine retard; LIFE 2001: losartan ± hydrochlorothiazide vs atenolol ± hydrochlorothiazide; MOSES 2005: eprosartan vs nitrendipine; NAVIGATOR 2010: valsartan vs placebo; PROFESS 2008: telmisartan vs placebo; RENAAL 2001: losartan vs placebo; SCOPE 2003: candesartan vs placebo; TRANSCEND 2008: telmisartan vs placebo; VALUE 2004: valsartan vs amlodipine.

The results for the mortality and morbidity endpoints are presented in *Figure 1* and *Table 1*. The IR of all-cause mortality was 0.0233/year in the patients randomized to RAAS inhibitors versus 0.0252 in controls (HR 0.95, 95% CI 0.91 to 0.99). The corresponding median NNT was 113 (2.5–97.5th percentile, 85 to 168), i.e. 113 (median value) patients would have to be treated with RAAS inhibitor for 4.3 years to prevent one death. When the analysis was performed separately for ACE inhibitors and ARBs, the ACE inhibitors reduced all-cause mortality (HR 0.90, 95% CI 0.84 to 0.97), while the ARBs had no effect (HR 0.99, 95% CI 0.94 to 1.04). The corresponding median NNT values were 67 (2.5–97.5th percentile, 53 to 92) for ACE inhibitors and 335 (2.5–97.5th percentile, – 4341 to 5076) for ARBs.

Similar results were found for cardiovascular mortality, with a median NNT value of 116

(2.5–97.5th percentile, 88 to 172) for ACE inhibitors versus 409 (2.5–97.5th percentile, – 3034 to 4431) for ARBs, and myocardial infarction, with a median NNT of 80 (2.5–97.5th percentile, 65 to 105) for ACE inhibitors versus 338 (2.5–97.5th percentile, – 4184 to 5427) for ARBs. For the two mortality endpoints and myocardial infarction, therefore, the advantage in NNT values for the RAAS inhibitors was driven by the ACE inhibitor trials.

The effect of RAAS inhibitors on incident stroke was statistically non-significant (HR, 0.91, 95% CI 0.83 to 1.00), whereas no differences were observed between ACE inhibitors and ARBs (HRs 0.92 and 0.90, respectively). The effect on the composite of myocardial infarction and stroke (HR, 0.93, 95% CI 0.87 to 0.99, NNT 136, 2.5–97.5th percentile 96–235) appeared to be driven by the effect of ACE inhibitors on myocardial infarction.

Complete results separated by individual trials are shown in the Supplementary material (**Webtable 2**).

Table 1. Effect of renin angiotensin aldosterone system (RAAS) inhibition on all-cause mortality, cardiovascular mortality, myocardial infarction, stroke, and a composite of myocardial infarction and stroke over 4.3 years.

	Incidence rate (events/patient-year)		HR (95% CI)	NNT (2.5th to 97.5th percentile)
	Control	Active		
All-cause mortality				
RAAS inhibitor	0.0252	0.0233	0.954 (0.91 to 0.99)	113 (85 to 168)
ACE inhibitor	0.0255	0.022	0.905 (0.84 to 0.97)	67 (53 to 92)
ARB	0.0249	0.0246	0.991 (0.94 to 1.04)	335 (– 4341 to 5076)
Cardiovascular mortality				
RAAS inhibitor	0.0117	0.0104	0.934 (0.87 to 1.00)	170 (126 to 259)
ACE inhibitor	0.012	0.0103	0.884 (0.78 to 1.01)	116 (88 to 172)
ARB	0.0111	0.0104	0.969 (0.90 to 1.05)	409 (– 3034 to 4431)
Myocardial infarction				
RAAS inhibitor	0.013	0.0107	0.956 (0.89 to 1.02)	117 (95 to 154)
ACE inhibitor	0.0148	0.012	0.921 (0.86 to 0.99)	80 (65 to 105)
ARB	0.0094	0.0092	1.004 (0.91 to 1.11)	338 (– 4184 to 5427)
Stroke				
RAAS inhibitor	0.0137	0.0125	0.912 (0.83 to 1.00)	203 (136 to 404)
ACE inhibitor	0.0092	0.009	0.923 (0.78 to 1.09)	337 (165 to 1752)
ARB	0.0196	0.0179	0.902 (0.80 to 1.01)	131 (83 to 308)
Composite of myocardial infarction and stroke				
RAAS inhibitor	0.0246	0.0227	0.927 (0.87 to 0.99)	136 (96 to 235)
ACE inhibitor	0.0233	0.0204	0.896 (0.80 to 1.01)	86 (64 to 131)
ARB	0.0267	0.025	0.947 (0.86 to 1.04)	157 (89 to 567)

Hazard ratios (HR) and number needed to treat (NNT, median value) with the corresponding 95% confidence intervals (CI). ACE = angiotensin-converting enzyme; ARB = angiotensin II receptor blocker. Incidence rate = number of events in the group / (mean follow-up duration × number of patients in group).

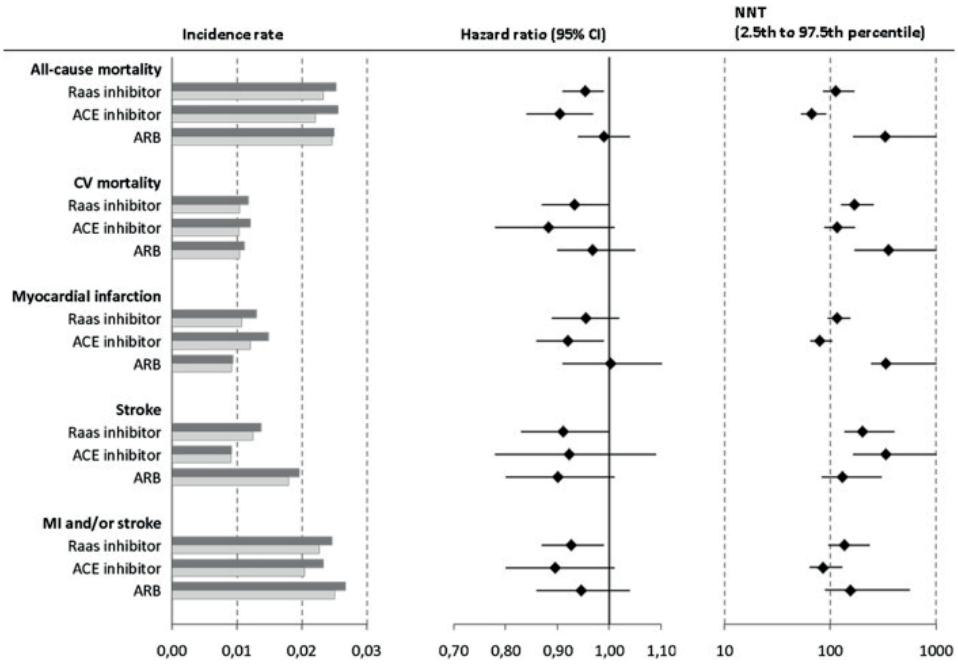


Figure 1. Effect of renin angiotensin aldosterone system (RAAS) inhibition on all-cause mortality, cardiovascular (CV) mortality, myocardial infarction (MI), stroke, and a composite of MI and stroke over 4.3 years: hazard ratios and number needed to treat (NNT, median value) with the corresponding confidence intervals (CI).
ACE=angiotensin-converting enzyme; ARB=angiotensin II receptor blocker.
Incidence rate = number of events in the group/(mean follow-up duration x number of patients in group). Dark grey bars, RAAS inhibitor, light grey bars, control.

Webtable 2. Individual trial results.

Trial	Type of RAAS inhibitor	Name	All-cause mortality		Cardiovascular mortality	
			HR (95% CI)	NNT (2.5-97.5th percentile)	HR (95% CI)	NNT (2.5-97.5th percentile)
RENAAL	ARB	Losartan	1.03 (0.83 to 1.29)	-28 (-674 to 534)		
IDNT	ARB	Irbesartan	0.92 (0.69 to 1.23)	26 (-660 to 638)	1.24 (0.87-1.75)	-35 (-437 to 376)
LIFE	ARB	Losartan	0.88 (0.77 to 1.01)	106 (-526 to 812)	0.87 (0.72-1.05)	156 (-1065 to 1624)
ALLHAT	ACE inhibitor	Lisinopril	1.03 (0.90 to 1.15)	-164 (-4607 to 4491)	1.02 (0.93-.1.12)	-270 (-5737 to 5475)
ANBP-2	ACE inhibitor	Enalapril	0.90 (0.75 to 1.09)	111 (-1706 to 1699)	1.01 (0.75-1.37)	-115 (-3885 to 3616)
SCOPE	ARB	Candesartan	0.96 (0.81 to 1.14)	79 (-1327 to 1542)	0.94 (0.75-1.18)	98 (-1659 to 1937)
Pilot HYVET	ACE inhibitor	Lisinopril	0.99 (0.62 to 1.58)	10 (-362 to 330)	1.00 (0.60-1.67)	9 (-361 to 411)
JMIC-B	ACE inhibitor	Ena/Lisi/Imi	1.32 (0.61 to 2.86)	-64 (-1193 to 1083)	1.08 (0.35-3.36)	-62 (-2242 to 2044)
VALUE	ARB	Valsartan	1.04 (0.94 to 1.14)	-158 (-2470 to 2358)	1.00 (0.86-1.18)	-148 (-5052 to 4498)
MOSES	ARB	Eprosartan	1.07 (0.73 to 1.57)	-30 (-628 to 612)		
ASCOT-BPLA	ACE inhibitor	Perindopril	0.89 (0.81 to 0.99)	154 (73 to 784)	0.77 (0.65-0.90)	157 (98 to 409)
ADVANCE	ACE inhibitor	Perindopril	0.86 (0.75 to 0.98)	91 (45 to 459)	0.82 (0.68-0.98)	121 (59 to 611)
HYVET	ACE inhibitor	Perindopril	0.79 (0.65 to 0.95)	24 (13 to 99)	0.78 (0.60-1.02)	37 (-130 to 261)
PRoFESS	ARB	Telmisartan	1.03 (0.93 to 1.14)	-126 (-2596 to 2620)	0.85 (0.71-1.02)	150 (-793 to 1108)
TRANSCEND	ARB	Telmisartan	1.05 (0.91 to 1.22)	-99 (-1852 to 1633)	1.02 (0.85-1.23)	-104 (-2465 to 2385)
CASE-J	ARB	Candesartan	0.85 (0.62 to 1.16)	103 (-1259 to 1506)		
HJ-CREATE	ARB	Candesartan	1.18 (0.83 to 1.67)	-67 (-1055 to 881)	1.12 (0.65-1.92)	-95 (-1960 to 2011)
NAVIGATOR	ARB	Valsartan	0.90 (0.77 to 1.05)	182 (-1886 to 2036)	1.11 (0.86-1.42)	-316 (-5193 to 4804)

Webtable 2 continued.

Trial	Type RAASi	Myocardial infarction		Stroke		Composite myocardial infarction/stroke	
		HR (95% CI)	NNT (2.5-97.5th percentile)	HR (95% CI)	NNT (2.5-97.5th percentile)	HR (95% CI)	NNT (2.5-97.5th percentile)
RENAAL	ARB	0.74 (0.52-1.07)	32 (-223 to 247)				
IDNT	ARB	1.18 (0.83-1.69)	-38 (-575 to 510)	1.29 (0.81-2.04)	-45 (-632 to 602)	1.22 (0.92-1.62)	-28 (-284 to 250)
LIFE	ARB	1.05 (0.86-1.28)	-196 (-3851 to 3381)	0.75 (0.63-0.89)	69 (44 to 163)	0.86 (0.76-0.98)	81 (42 to 379)
ALLHAT	ACEi	0.97 (0.90-1.06)	257 (-4819 to 45980)	1.15 (1.03-1.28)	-185 (-733 to -99)	1.03 (0.97-1.10)	-206 (-3162 to 3026)
ANBP-2	ACEi	0.70 (0.50-0.98)	116 (54 to 701)	0.92 (0.74-1.15)	125 (-2002 to 2233)	0.85 (0.70-1.02)	79 (-292 to 581)
SCOPE	ARB	1.10 (0.78-1.54)	-141 (-2883 to 2473)	0.76 (0.58-1.01)	81 (-160 to 570)	0.88 (0.71-1.09)	87 (-1021 to 1184)
Pilot HYVET	ACEi			0.95 (0.48-1.91)	17 (-482 to 496)		
JMIC-B	ACEi	0.88 (0.42-1.83)	56 (-1244 to 1172)	1.08 (0.54-2.16)	-50 (-1275 to 1231)	0.98 (0.59-1.62)	31 (-980 to 960)
VALUE	ARB	1.18 (1.02-1.38)	-13 (-66 to -6)	1.15 (0.98-1.35)	-178 (-1384 to 848)	1.17 (1.05-1.30)	-84 (-274 to -48)
MOSES	ARB	0.80 (0.52-1.22)	33 (-422 to 478)	0.75 (0.58-0.97)	16 (8 to 79)	0.76 (0.61-0.95)	14 (7 to 60)
ASCOT-BPLA	ACEi	0.88 (0.76-1.00)	223 (-559 to 1553)	0.77 (0.67-0.89)	132 (85 to 298)	0.82 (0.75-0.91)	87 (57 to 183)
ADVANCE	ACEi	0.90 (0.76-1.06)	160 (-1647 to 1823)	0.98 (0.81-1.18)	179 (-4358 to 4556)	0.93 (0.82-1.05)	132 (-1335 to 1825)
HYVET	ACEi	0.71 (0.30-1.70)	153 (-2553 to 2568)	0.70 (0.49-1.01)	47 (-96 to 308)	0.71 (0.51-0.99)	41 (17 to 244)
PRoFESS	ARB	1.00 (0.81-1.24)	130 (-5211 to 5162)	0.95 (0.86-1.04)	109 (-1004 to 1349)	0.95 (0.88-1.04)	106 (-1327 to 1471)
TRANSCEND	ARB	0.79 (0.62-1.01)	107 (-315 to 733)	0.83 (0.65-1.06)	128(-1086 to 1297)	0.81 (0.68-0.97)	65 (34 to 305)
CASE-J	ARB	0.94 (0.49-1.83)	182 (-4866 to 4127)	1.22 (0.84-1.77)	-124 (-1732 to 1583)	1.14 (0.83-1.58)	-116 (-1985 to 1686)
HJ-CREATE	ARB	1.11 (0.66-1.89)	-94 (-2019 to 1823	0.92 (0.61-1.37)	75 (-1642 to 1594)	0.99 (0.71-1.36)	46 (-1331 to 1470)
NAVIGATOR	ARB	0.99 (0.78-1.25)	214 (-6967 to 6504)	0.80 (0.62-1.03)	238 (-1126 to 1723)	0.90 (0.75-1.06)	196 (-2033 to 2065)

ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker. RAAS, renin angiotensin aldosterone system. HR, hazard ratio. CI, confidence interval. NNT, number need to treat. Details of trials: ADVANCE 2007: perindopril/indapamide vs placebo; ALLHAT 2002: lisinopril vs chlorthalidone or amlodipine; ANBP-2 2003: enalapril or other ACE inhibitor vs hydrochlorothiazide or other diuretic; ASCOT-BPLA 2005: amlodipine ± perindopril vs atenolol ± bendroflumethiazide; CASE-J 2008: candesartan vs amlodipine; HIJ-CREATE 2009: candesartan vs non-ARB; HYVET 2008: indapamide ± perindopril vs placebo; Pilot HYVET 2003: lisinopril or other ACE inhibitor vs bendroflumethiazide (or other diuretic) or no treatment; IDNT 2001: irbesartan vs amlodipine or placebo; JMIC-B 2004: ACE inhibitor vs nifedipine retard; LIFE 2001: losartan ± hydrochlorothiazide vs atenolol ± hydrochlorothiazide; MOSES 2005: eprosartan vs nitrendipine; NAVIGATOR 2010: valsartan vs placebo; PROFESS 2008: telmisartan vs placebo; RENAAL 2001: losartan vs placebo; SCOPE 2003: candesartan vs placebo; TRANSCEND 2008: telmisartan vs placebo; VALUE 2004: valsartan vs amlodipine.

Discussion

A large body of evidence has emerged from randomized controlled trials of antihypertensives in head-to-head comparisons. Meta-analysis is a useful method to summarize the almost overwhelming wealth of data, and is important to support healthcare decisions by regulatory agencies, inform expert bodies developing guidelines, and help decision-making in clinical practice. In this paper, we have presented an effectiveness of ACEi versus ARB in hypertension using NNT data in a pooled analysis of 18 different RAAS inhibitor trials.[6] We found a median NNT of 67 patients to prevent one all-cause mortality event for ACE inhibitors during 4.3 years of treatment, and a (non-significant) 335 for the ARBs. Within the class of RAAS inhibitors, the NNT values for the mortality endpoints and the endpoints including myocardial infarction were systematically smaller for ACE inhibitors and systematically larger for ARBs. The difference between the two classes was less evident for stroke. Our findings are particularly significant since our analysis included only modern hypertension trials that enrolled patients already receiving background medications such as statins and antiplatelet agents. This underlines how use of ACE inhibitors in mild-to-moderate hypertension may still lead to additional treatment benefits in terms of a reduction in all-cause and cardiovascular mortality. Our results are consistent with previous reports of a neutral effect of ARBs on mortality[16] and with reports of reductions in mortality with ACE inhibitors.[17-20] They are also in line with other meta-analyses in other patient profiles.[8-11]

The most effective treatments have the lowest NNTs. The question of how low is low depends on the target of treatment as well the prevalence of the disease (worldwide). For instance, the prevention of vascular death in the first 5 weeks post-myocardial infarction with aspirin and streptokinase is associated with NNT values of between 20 and 40.[21] In long-term cardiovascular disease prevention, where patients already receive multiple medications to address their risk factors, higher values are usually reported whether it is for reduction in cardiovascular events or prevention of mortality. Indeed, the treatment of dyslipidemia in primary prevention for patients with multiple atherosclerotic risk factors – a strategy that is currently widely applied in clinical practice – was associated with a NNT of 190 over 4.8 years for all-cause mortality.[22] Statin treatment in men with hypercholesterolemia yielded an NNT of 112 over 4.9 years for all-cause mortality, an NNT of 44 for fatal and non-fatal coronary events, and 642 for stroke.[23] Therefore, in the population receiving more often contemporary treatments analyzed here, the NNTs for ACE inhibitors of 67 over 4.3 years for all-cause mortality and 116 for cardiovascular mortality are very significant, and should be considered together with the other benefits

associated with ACE inhibitors in the treatment of hypertension (i.e. reductions in morbidity). These benefits are essentially driven by a reduction in myocardial infarction and stroke as demonstrated by an NNT of 86 for the composite of the two endpoints in our analysis. Previous meta-analyses have indeed already reported an intrinsic benefit of ACE inhibitors on coronary events.[24-26] Indeed, regression analysis showed that even with no BP reduction, ACE inhibitors already provide significant reduction of coronary events.[26] However, the higher NNT of 338 to prevent one MI with ARBs reflects the controversy about cardiac benefits with this class. Indeed, in a 2006 meta-analysis, Strauss et al. reported a significant increase in myocardial infarction with ARBs that they qualified as the "ARB MI paradox".[24] They hypothesized that various biological parameters could explain the differences in cardioprotection between ACE inhibitors and ARBs. The most plausible seems to be the uncovered stimulation of angiotensin II receptors due to increased circulating angiotensin II during ARB treatment, which could cause endothelial cell inflammation and apoptosis. On the other hand, ACE inhibitors have the advantage of preserving bradykinin and may therefore enhance its protective effects against ischemia and vascular injury. It now appears clear that these differences of mode of action have consequences beyond MI protection and translate into important differences in terms of mortality benefits above the mere blood-pressure effect of both agents. Considering the endpoint stroke, ARB tended to have a lower NNT as compared to ACE inhibitors in our analysis, although differences were non-significant. The specific blood pressure lowering effect of ARBs could explain this result. Pleiotropic effects above a mere blood-pressure lowering effect could be the basis of the beneficial effects on mortality and MI for the ACE-inhibitors. Most importantly, the observed heterogeneous effects underline that the agents are not equal, which is relevant for the still ongoing ACE inhibitor versus ARB debate with respect to hypertensive patients.

Our analysis is not without limitations. The meta-analysis does not contain the individual trial data of the 18 included trials to perform adjustments for confounding factors or subgroup analyses. Additionally, we cannot exclude an underestimation of the IRs, though this would apply in the both active treatment and control groups, and may therefore not have a major effect on the comparison. Next, NNTs are sensitive to differences in baseline risk. Mortality rates were similar in control patients in the ACE inhibitor and ARB trials. NNT values also depend on the duration of follow-up. Our results were therefore standardized for the mean duration of follow-up of the 18 studies included in the analysis. The standardization of follow-up duration assumes that the baseline rates and the effect of treatment are constant over the range of follow-up times considered (1.1 to 6.1 years).[27, 28] However, analysis of available Kaplan Meier curves suggests that over the considered period of time, mortality rates and treatment

effects were roughly linear,[2,4,29-32] and that this therefore is a reasonable assumption. Finally, in the context of a clinical trial, myocardial infarction events are first events, and so our analysis could not be applied to repeated myocardial infarction events.

Although NNTs present treatment effects in a clinically relevant way, they have been criticized as misleading because they suggest an "individualization of the benefit". Indeed, the wording associated with NNTs implies that one patient will avoid the event by treating NNT patients and that conversely, NNT-1 patients will not benefit from treatment. Negative NNTs are difficult to interpret implying the possibility of harm by treatment. In fact, it is most likely that all patients benefit a little from the treatment. Thus, although NNTs present data in a simple way, caution should be exercised not to misinterpret the data.

Conclusions

Among hypertensive patients at low to moderate risk, ACE inhibitors, but not ARBs, have a substantial absolute benefit in terms of reduction of both all-cause and cardiovascular mortality as well as myocardial infarction. These findings suggest that ACE inhibitors should be preferred in the management of hypertension and the agents cannot be considered as mere equivalents.

Conflict of interest

None. This combined analysis was initiated by the authors and was designed, conducted, interpreted, and reported independently. The current study had no funding source or any with a participating role in data collection, outcome assessment. All authors had joint responsibility for the decision to submit for publication.

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Contribution of authors

All authors contributed to the analysis of the data and writing of the report. All authors

approved the final version of the manuscript. JB writing manuscript & concept, LvV writing, data collection and statistics, KMA writing, MB writing, KF writing, JJM writing & concept, EB. Data collection and statistical analysis & concept. All authors approved the final version of the manuscript after critical appraisal.

QUOROM/PRISMA guidelines

The article is written according to the quorum/prisma guidelines for meta-analyses of RCTs as described in Webfigure 1. For all included RCT's, already published, the appropriate references are noted.

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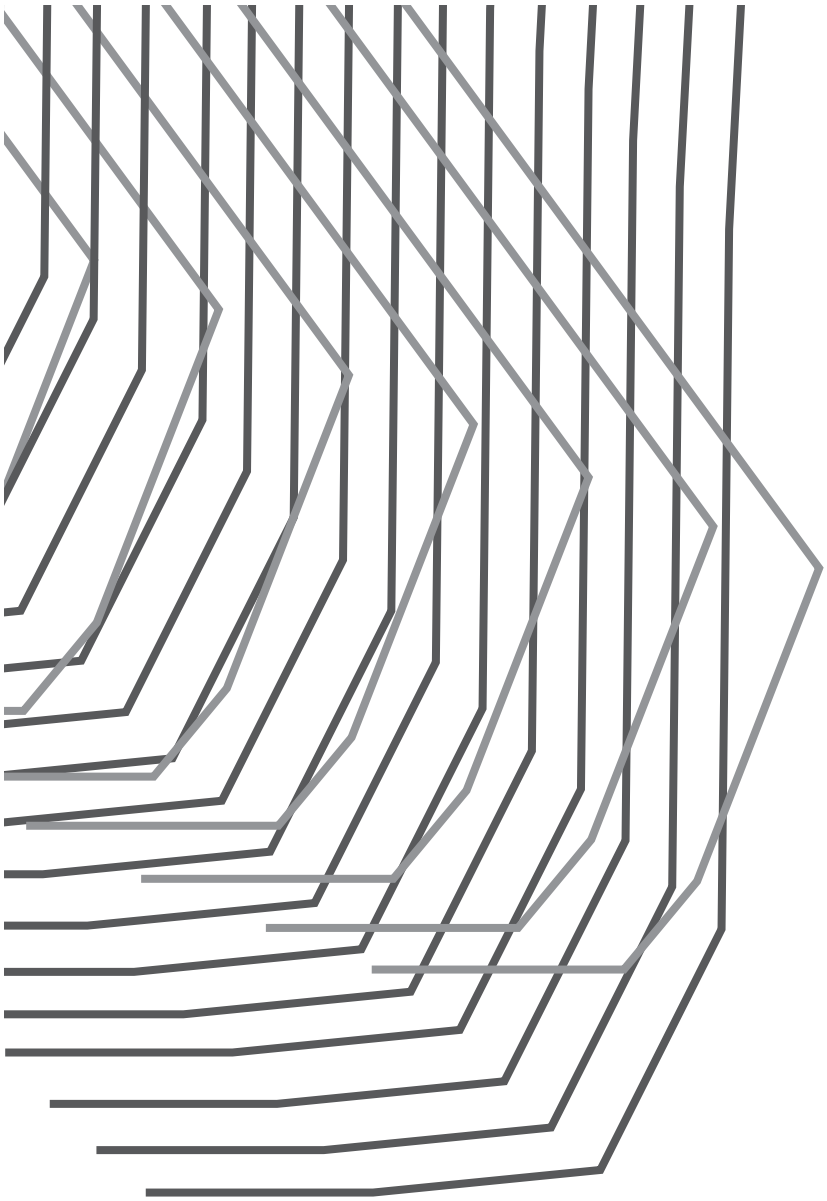

Chapter 13

Summarizing discussion

Summarizing discussion

Biomarkers in heart failure

Heart failure (HF) is highly prevalent. Due to the increasing burden of HF risk factors, increasing age and improved treatment of cardiovascular disease the prevalence of HF still increases. It is estimated that the lifetime risk for developing HF, ranges between 20% and 46% in middle-aged men and women.[1] Guidelines emphasize the need for identifying individuals that are at increased risk for developing HF, which is still difficult.[2] Biomarkers may aid in selecting individuals that are at risk for developing HF or for progression of HF.[3]

The TRanslational Initiative on Unique and novel strategies for Management of Patients with Heart failure (TRIUMPH) study was designed to evaluate the prognostic properties of serially-measured biomarkers using a unique design of seven planned repeated measurements during one year follow-up in acute HF patients. [4] Because of this design, we were able to take into account the change in biomarker levels over time representing the dynamic nature of HF.[3] Patients were eligible for enrolment if they were hospitalized with decompensation of known chronic HF or newly diagnosed HF. TRIUMPH was a multi-centre observational cohort study, with 14 hospitals in the Netherlands enrolling 496 patients, between September 2009 and December 2013.

In **part I** of this thesis, **chapter 2,** we evaluated the prognostic properties of galectin-3 in acute HF patients in the TRIUMPH cohort. The baseline galectin-3 level was associated with an increased risk of reaching the primary endpoint (composite of all-cause mortality and readmission for HF during one year follow-up), as well as with the secondary endpoints all-cause mortality, cardiovascular mortality, and HF readmission. After adjustment for all selected potential confounders (age, sex, systolic blood pressure, diabetes mellitus, left ventricular ejection fraction (LVEF), previous hospitalization for HF during the last 6 months, ischemic HF, body mass index, estimated glomerular filtration rate and baseline NT-proBNP level) the strength of the association between baseline galectin-3 and the different endpoints became weaker, and only the association with all-cause mortality remained statistically significant. On the other hand, when repeated measurements were taken into account, galectin-3 showed to be a predictor of outcome in acute HF patients, independent of potential confounders including repeated measurements of NT-proBNP. Moreover, the average galectin-3 levels increased in patients who would subsequently reach an adverse outcome. Hence, galectin-3 may be helpful for prognostication in clinical practice, especially when measured repeatedly during follow-up while hospitalized and at the outpatient clinic.

A second promising biomarker evaluated in the TRIUMPH cohort is ST2 (**chapter 3 and 4**). We demonstrated that repeated measurements of ST2 are a strong and independent predictor of adverse outcome during one year follow-up in patients following admission for acute HF. Repeated ST2 measurements offer substantial and incremental prognostic value to that conferred by other known risk factors and, importantly, repeated measurements of NT-proBNP. Another finding of the present study is that the estimated average ST2 levels appeared to increase in patients prior to reaching the primary endpoint, whereas the average estimated ST2 level in patients without the primary endpoint during follow-up stabilizes. Furthermore, in a post hoc analysis, we defined ST2 patterns in individual patients. We demonstrated that 82% of patients with a so-called "J-shaped" ST2 pattern remained event-free during 1 year of follow-up, while the overall percentage of patients in the TRIUMPH cohort reaching the primary endpoint was 40%. These results suggest that repeated ST2 measurements in addition to NT-proBNP measurements may be helpful in clinical practice to identify HF patients who are at increased risk of adverse outcome.

In a scientific statement of the American Heart Association, it is proposed that using a multi biomarker strategy may be useful for providing additional risk stratification. [5] In **chapter 5** we performed a multi marker and moreover a multi time point analysis, with repeated biomarker measurements during 1 year of follow-up in the TRIUMPH cohort. We demonstrated that repeated measurements of NT-proBNP, ST2, galectin-3, cTnI and creatinine are a strong and independent predictor of all-cause mortality. Our results show that combining serial measurements of NT-proBNP and ST2 offer substantial independent prognostic value. Additionally, this study suggests that multiple measurements are helpful to identify patients with an increased biomarker level and therefore are at increased risk of death at a certain point in time. Future studies should evaluate the value of repeated biomarker measurements when used to guide treatment decisions. This is important, as it is proposed that biomarkers are useful in clinical practice, if they meet three criteria. (1) A clinician should be able to accurately measure a biomarker, (2) a biomarker should add new information and (3) a biomarker should help clinicians to manage patients.[6] Additional studies should also determine the number and timing of biomarker measurements needed for optimal prognostication and therapy monitoring.

Besides the traditional blood biomarkers, microRNA's have been proposed as an attractive new class of biomarkers.[7] We evaluated several microRNAs in the TRIUMPH cohort **(chapter 6)**. Direct RNA sequencing of plasma from instrumented pigs revealed a number

of circulating microRNAs to be produced by the pig myocardium, including miR-1306-5p, which had not yet been identified as a microRNA related to the heart. Subsequently, we demonstrated that miR-1306-5p was positively and independently associated with all-cause mortality and HF hospitalization. Other microRNAs known to be cardiac-enriched or previously linked to HF were also evaluated in the TRIUMPH cohort. Associations of temporal patterns of miR-320a, miR-378a-3p, miR-423-5p and miR-1254 with adverse clinical outcomes were, however, not independent of clinical characteristics. We found that myocyte-specific microRNAs were non-detectable in a large proportion of samples. In future, more sensitive myocyte-specific microRNA assays are needed to allow precise estimations of the risk associated with elevated levels of these microRNAs, and to investigate whether they are capable of providing additional information to established predictors of clinical risk.

In **chapter 7** we showed that lipoprotein-associated phospholipase A2 (Lp-PLA2), a biomarker related to vascular inflammation, is a predictor of incident HF. Lp-PLA2 activity was determined in a random sample of 1820 subjects from the Rotterdam Study, a population-based cohort study among persons 55 years and over.[8] During a mean follow-up of 6.7 years, 94 HF cases occurred. We excluded participants with HF of coronary heart disease at baseline and we accounted for incident coronary heart disease during follow-up. We showed that Lp-PLA2 activity was independently associated with incident HF. Recently, the treatment effect of darapladib, a selective Lp-PLA2 inhibitor, was evaluated in a randomized controlled trial, in stable coronary heart disease patients.[9] Darapladib reduced Lp-PLA2 activity, but did not significantly reduce cardiovascular events. Still baseline Lp-PLA2 was found to be independently associated with cardiovascular events and HF hospitalization. In future, research might be performed to assess the treatment benefits of Lp-PLA2 inhibitors in a HF population. For now, Lp-PLA2 seems to be a biomarker for prognostication.[10,11]

Besides being highly prevalent, HF is also a complex and progressive disease. Several pathophysiological mechanisms are present in HF and interact with each other.[3] HF is dynamic and progresses over time and its phenotype is diverse. Classically, HF was considered to be associated with impaired cardiac contractility and cardiac dilatation. In the past decade, however, it has become evident that a considerable portion of patients presenting with clinical HF have a normal left ventricular ejection fraction (LVEF). Some studies report a prevalence of HF with a normal LVEF as high as 50%.[12] In the 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure, different types of HF have been described, based on LVEF measurements.[13] These types are HF with reduced ejection fraction (HFrEF), HF with mid-range ejection

fraction (HFmrEF) and HF with a preserved ejection fraction (HFpEF). Although it is not clear whether these different HF types represent different syndromes or whether they are part of one HF spectrum, it is known that demographics, co-morbidities and response to treatment is different.[12] Biomarker levels are also different in HFrEF and HFpEF patients.[14] We performed a systematic review on biomarkers in HFpEF patients and found that several biomarkers and biomarker categories, including biomarkers of myocyte stress, inflammation, extracellular matrix remodelling, GDF-15, cystatin C, resistin, and galectin-3, appear to be potentially promising diagnostic tools in HFpEF. Some of them, including TNF-α, IL-6, PINP, PIIINP, osteopontin, and cystatin C, may carry prognostic value as well (**chapter 8**). Further research may provide additional evidence for the value of these biomarkers in improvement of risk stratification of patients with HFpEF. Applying a multiple biomarker strategy may result in even further improvement of risk stratification compared with using one biomarker alone.[13]

Health related quality of life in heart failure patients

Within the TRIUMPH cohort, we evaluated co-morbidities, symptom burden and health related quality of life (HR-QoL), **part II** of this thesis. There is not a gold standard for measuring HR-QoL or symptom burden in HF patients.[15] To account for this limitation, we used not 1 but a set of 4 questionnaires. Therefore, patients were asked to complete several questionnaires at discharge and after 9-12 months of follow-up. HR-QoL was assessed using a specific HF-related questionnaire, the Kansas City Cardiomyopathy Questionnaire (KCCQ), and a non-disease specific questionnaire, the EuroQol 5 Dimensions (EQ-5D). Furthermore, depressive symptoms and anxiety were measured with the Hospital Anxiety and Depression Scale (HADS) and finally symptom burden was assessed by a questionnaire on symptom occurrence and symptom burden.

Chapter 9 demonstrates that patients with HF report a high symptom occurrence and a high symptom burden, which is in line with previous research. Moreover, in clinical practice it is known that symptoms are under-recognized and therefore undertreated, despite the predictable pattern of progression of HF.[16] This supports our recommendation that elaborate research and education on symptom burden is highly important. Furthermore, we determined that symptom burden was even worse in patients with depression compared to those without depression. Therefore, clinicians should give even more attention to symptom management in patients with depression. Also, recognition and treatment of depression in HF patients deserves attention.[17]

Furthermore, in **chapter 10,** we evaluated the relationship between comorbidities and HR-QoL and depression. In the TRIUMPH cohort, HF patients without comorbidity (prior

CVA, chronic kidney dysfunction, diabetes and COPD) had better HR-QoL and less depression compared to patients with comorbidity. This finding supports the statement that in order to improve HR-QoL, HF guideline-driven care should also include optimal management of the most prevalent non-cardiovascular comorbidities.[18] Furthermore, determinants related to a worse HR-QoL were sex, history of HF, BMI, NT-proBNP at admission, systolic blood pressure at discharge and the presence of a depression. Although most of these factors have been associated with HR-QoL previously, interestingly, the association between these characteristics and HR-QoL were merely found in the subgroup of patients with comorbidity.[19]

Future research is necessary to establish how to improve symptom recognition and treatment in HF patients. We should prioritize our aim to reduce symptoms in HF patients and, hence, improve HR-QoL, which, for many patients may be felt as important as the crude life-expectancy.[20,21] Symptom burden, together with depression and co-morbidities seem to be important targets in successfully treating HF patients.

RAAS inhibitors and outcome in hypertensive patients

In **part III** of this thesis, **chapter 11,** we performed a meta-analysis, which included almost 160 000 patients, and showed that renin-angiotensin-aldosteron system (RAAS) inhibitors are associated with a 5% reduction in all-cause mortality during a 4-year follow-up period in hypertensive patients. To our knowledge, we were the first to perform a meta-analysis investigating the efficacy of RAAS inhibitors on all-cause and cardiovascular mortality in their main indication of hypertension. The findings are relevant to clinical practice, as they are based on data from well-designed randomized trials encompassing a broad population of patients with high blood pressure, who were well-treated for concomitant risk factors and who represent usual hypertensive patients seen today.

The clinically most important pharmacologic agents that block the RAAS currently are the angiotensin-converting enzyme (ACE) inhibitors and AT1 receptor blockers (ARBs). Although ACE inhibitors and ARBs are both RAAS inhibitors, they have different mechanisms of action.[22] We performed a post-hoc stratified analysis and determined that the observed overall all-cause 5% mortality reduction resulted almost completely from the beneficial effect of the class of ACE inhibitors (10% relative reduction in all-cause mortality), whereas the ARBs showed a neutral treatment effect with respect to all-cause mortality. It should be emphasized that we did not design this meta-analysis to make this head-to-head comparison between ACE inhibitors and ARBs.

The 2018 ESC/ESH "Management of arterial hypertension" guideline states that ACE

inhibitors and ARBs have similar effectiveness.[23] This statement is primarily based on a meta-analysis performed in 2008 by Reboldi.[24] Interestingly, the trials included in that meta-analysis did not primarily consist of hypertensive patients per se, with prevalence of hypertension varying from 35% to 100%. Li et al, performed a meta-analysis comparing the effect of ACE inhibitors and ARBs in strictly hypertensive populations.[25] Subgroup data, of hypertensive patients, were used if trials included patients with and without hypertension. This meta-analysis included randomized controlled trials that compared ACE inhibitors and ARBs in a head-to-head manner and showed no difference for total mortality (moderate-quality evidence) and total cardiovascular events (low-quality evidence). The authors state that the results were mainly driven by a single large study, which merely included patients with confirmed vascular disease or end-organ damage, which makes the results of the meta-analysis less generalizable to an asymptomatic hypertensive population.[26]

There is no profound evidence that a different treatment effect exists between ACE inhibitors and ARBs in hypertensive patients. On the contrary, there are no randomized controlled trials presented comparing ARBs to placebo in hypertensive patients, which do exist for ACE inhibitors.[27] Therefore, the less established degree of evidence together with our post-hoc analyses may favor the use of ACE inhibitors in treating hypertension.

In **chapter 12**, we performed a 'number needed to treat' (NNT) analysis to give insight into the absolute treatment effect of RAAS inhibitors in hypertensive patients instead of relative risk reduction alone. The NNT is considered an important measure to accurately communicate risk.[28, 29] The corresponding median NNT to prevent one death was 113 (2.5–97.5th percentile, 85 to 168) in favour of RAAS inhibitors, compared to placebo or other blood pressure lowering drugs, during 4 years of follow-up. We also assessed the effectiveness of ACE inhibitors and ARBs separately, for the prevention of additional endpoints, including cardiovascular death, myocardial infarction and stroke in hypertensive patients. ACE inhibitors reduce all-cause mortality (corresponding NNT 67), cardiovascular mortality (NNT 116) and myocardial infarction (NNT 80). The corresponding NNT during 4 years of treatment for ARBs in hypertensive patients were higher and non significant (NNT 335 for all-cause mortality, NNT 409 for cardiovascular mortality and NNT 338 for myocardial infarction). The treatment effect of RAAS inhibitors on the incidence of stroke was similar for ACE inhibitors and ARBs.

The most effective treatments have the lowest NNT. The clinical implication related to the NNT depends on several parameters, such as treatment outcome, treatment duration, treatment cost, treatment side effects, absolute baseline risk, as well as the prevalence

of the disease.[28, 30] Hypertension is highly prevalent in the general population [31] and taking into account the relatively low absolute mortality risk in this population, reaching a NNT of 113 to prevent one death during 4 years of treatment seems to be a relevant treatment effect. Furthermore, the added morbidity gain, preventing myocardial infarction and stroke, should also encourage physicians to treat hypertensive patients with a RAAS inhibitor.

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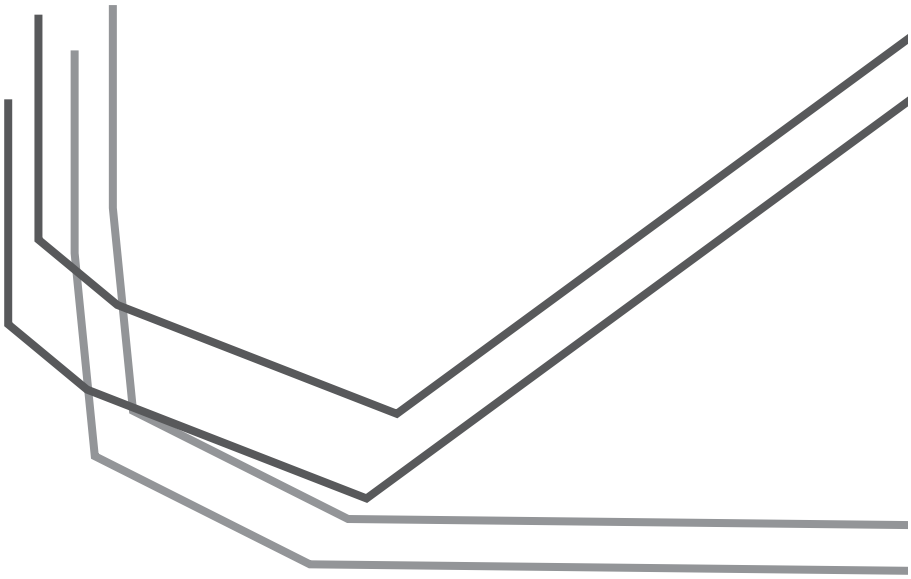
Nederlandse samenvatting



Nederlandse samenvatting

Biomarkers en hartfalen

Hartfalen komt veel voor en het aantal mensen met hartfalen groeit gestaag. De oorzaken hiervan zijn onder andere een toename van risicofactoren voor hartfalen binnen de algemene bevolking, een toenemende vergrijzing en de verbeterde behandeling van hart- en vaatziekten. Naar schatting is het risico op het ontwikkelen van hartfalen gedurende het gehele leven bij mannen en vrouwen van middelbare leeftijd tussen de 20% en 46%.[1] Richtlijnen benadrukken de noodzaak individuen met een verhoogd risico op het ontwikkelen van hartfalen te identificeren.[2] Bij het selecteren van deze personen zouden biomarkers kunnen helpen. Hiernaast zouden biomarkers kunnen vast stellen welke hartfalen patiënten risico lopen op klinische progressie van de ziekte.[3]

De 'TRanslational Initiative on Unique and new strategies for Management of Patients with Heart failure' (TRIUMPH) studie is opgezet om de prognostische waarde van serieel gemeten biomarkers te evalueren bij patiënten die opgenomen werden met acuut hartfalen. Er werd gebruik gemaakt van een uniek ontwerp waarbij zeven herhaalde metingen gepland werden gedurende één jaar.[4] Vanwege deze herhaalde metingen zien we de verandering in biomarker waarde binnen een patiënt uitgezet in de tijd. Dit biomarker beloop is een goede weergave van het dynamische ziekteproces bij patiënten met hartfalen.[3] Patiënten kwamen in aanmerking voor deze studie als ze in het ziekenhuis werden opgenomen met acuut gedecompenseerd hartfalen. Patiënten konden opgenomen worden met 'de novo' hartfalen of al bekend zijn met hartfalen. TRIUMPH is een observationele multicenter cohort studie, waarbij 14 Nederlandse ziekenhuizen 496 patiënten hebben geïncludeerd, tussen september 2009 en december 2013.

Deel I van dit proefschrift, is gericht op de prognostische waarde van verschillende biomarkers in patiënten met acuut hartfalen, binnen het TRIUMPH-cohort. Galectine-3 is één van de biomarkers die onderzocht is **(hoofdstuk 2)**. Een enkele galectine-3 meting tijdens de initiële opname is geassocieerd met zowel het primaire eindpunt (combinatie van sterfte of ziekenhuis opname voor acuut hartfalen) en de individuele secundaire eindpunten (sterfte, cardiovasculaire sterfte en ziekenhuis opname voor acuut hartfalen). Nadat rekening gehouden werd met mogelijk beïnvloedende factoren (leeftijd, geslacht, systolische bloeddruk, aanwezigheid van diabetes mellitus, linker ventrikel ejectie fractie, eerdere ziekenhuisopname voor hartfalen gedurende de afgelopen 6 maanden, ischemische hartfalen, lichaamsgewicht index, nierfunctie en NT-proBNP waarde) werd de associatie tussen een enkele galectine-3 meting en de verschillende eindpunten

zwakker. Alleen de associatie tussen de galectine-3 meting en het eindpunt sterfte bleef statistisch significant. Echter, wanneer alle serieel gemeten galectine-3 waarden werden geanalyseerd, bleef galectine-3 weldegelijk een significante voorspeller voor de verschillende eindpunten, onafhankelijk van de mogelijke beïnvloedende factoren en serieel gemeten NT-proBNP waarden. Hierbij concluderen wij dat galectine-3 een goed hulpmiddel zou kunnen zijn, naast NT-proBNP, om te kunnen evalueren welke hartfalen patiënten een slechtere prognose hebben. Vooral wanneer galectine-3 herhaaldelijk gemeten wordt tijdens een opname en gedurende het poliklinische vervolg.

ST2 is een tweede veelbelovende biomarker die onderzocht is in het TRIUMPH-cohort (**hoofdstuk 3 en 4**). We hebben aangetoond dat bij patiënten opgenomen met acuut hartfalen, serieel gemeten ST2 waarden een voorspeller is van de verschillende eindpunten. Deze associatie is onafhankelijk van bekende risicofactoren en serieel gemeten NT-proBNP waarden. Daarnaast hebben we onderzocht wat het beloop is van de gemiddelde ST2 waarde gedurende één jaar. We hebben gevonden dat de gemiddelde ST2 waarde van de patiënten die tijdens het jaar een eindpunt bereikten toenam voorafgaande aan dit eindpunt. Daarentegen bleef de gemiddelde ST2 waarde van de patiënten die tijdens het jaar geen eindpunt bereikten laag. Vanwege deze observatie hebben we een post-hoc analyse uitgevoerd waarbij we de serieel gemeten ST2 waardes van individuele patiënten hebben uitgezet tegen de tijd. Vervolgens hebben we per patiënt het ST2 patroon ingedeeld in een “U-vorm” dalende ST2 waarde na inclusie en stijging nadien, een “J-vorm” dalende ST2 waarde na inclusie met aansluitend stabilisatie of een ander patroon. Hierbij zagen we dat 82% van de patiënten met een zogenaamde “J-vorm” gedurende het vervolg van de studie geen eindpunt bereikten. Gemiddeld genomen bereikte 40% van de patiënten in het TRIUMPH-cohort het primaire eindpunt. Deze resultaten suggereren dat serieel gemeten ST2 waarden naast serieel gemeten NT-proBNP waarden geschikt kunnen zijn om hartfalen patiënten te identificeren met een verhoogd risico op een slechte uitkomst.

In een wetenschappelijke verklaring van de “American Heart Association” wordt voorgesteld dat het combineren van meerdere biomarkers nuttig kan zijn voor aanvullende risicostratificatie.[5] In **hoofdstuk 5** hebben we dan ook in het TRIUMPH-cohort seriële metingen van verschillende biomarkers gecombineerd in één analyse. We hebben aangetoond dat serieel gemeten NT-proBNP, ST2, galectine-3, cTnI en creatinine waarden een sterke en onafhankelijke voorspeller zijn van sterfte. Onze resultaten tonen aan dat bovenal het combineren van serieel gemeten NT-proBNP en ST2 waarden belangrijke onafhankelijke voorspellers zijn van (cardiovasculaire) sterfte. Bovendien suggereren de resultaten van deze analyse dat seriële metingen een

belangrijke bijdrage kunnen leveren aan het identificeren van patiënten wiens risico op overlijden toe neemt gedurende opname en poliklinische controle.

Toekomstige studies zouden het belang van seriële biomarker metingen verder moeten uitdiepen, waarbij een belangrijk vraagstuk is of de biomarker waarden gebruikt kunnen worden voor het nemen van behandel beslissingen. Het beantwoorden van deze vraag is van belang, omdat biomarkers pas daadwerkelijk geschikt zijn in de klinische praktijk, als ze aan drie criteria voldoen. (1) Een clinicus moet een biomarker nauwkeurig kunnen meten, (2) een biomarker moet nieuwe informatie toevoegen (3) een biomarker moet clinici helpen behandel beslissingen te nemen.[6] Daarnaast is het van belang kennis te verkrijgen over de timing en het aantal benodigde biomarker metingen voor het optimaal vast stellen van de prognose en het monitoren van de behandeling.

Naast de traditionele biomarkers is er een belangrijke nieuwe klasse biomarkers, de microRNA's,[7] waarvan we er verschillende in het TRIUMPH-cohort hebben geëvalueerd **(hoofdstuk 6).** In varkensmodellen zijn een aantal microRNA's geïdentificeerd, die geproduceerd worden in het myocard van varkens met hartfalen. Een van deze microRNA's is miR-1306-5p, welke voor zover wij weten, nog niet eerder gerelateerd is aan hartfalen. Vervolgens hebben we miR-1306-5p onderzocht in het TRIUMPH cohort, en hebben wij een onafhankelijk relatie gevonden met het primaire eindpunt (combinatie van sterfte of ziekenhuis opname voor acuut hartfalen). Vervolgens hebben we ook andere microRNA's geëvalueerd in het TRIUMPH cohort. Deze microRNA's werden ofwel geproduceerd in het myocard of zijn al in eerdere studies geassocieerd met hartfalen. De associaties van deze microRNA's (miR-320a, miR-378a-3p, miR-423-5p en miR-1254) met de verschillende eindpunten waren echter niet onafhankelijk van klinische factoren. Wat deze resultaten waarschijnlijk beïnvloed hebben, is dat de myocyt-specifieke microRNA's in een groot deel van de bloedmonsters niet detecteerbaar was. Het is dan ook noodzakelijk dat in de toekomst meer gevoelige myocyt-specifieke microRNA-testen ontwikkeld worden, waarna een associatie tussen de microRNA's en het risico op eindpunten beter onderzocht kan worden in bloedmonsters van hartfalen patiënten.

In **hoofdstuk 7** hebben we aangetoond dat lipoproteïne-geassocieerd fosfolipase A2 (Lp-PLA2), geassocieerd is met het ontwikkelen van hartfalen. Lp-PLA2 activiteit werd bepaald in 1820 proefpersonen, een steekproef uit de Rotterdam Studie, dit is een cohort studie onder 55-plussers die in de wijk Ommoord wonen in Rotterdam.[8] Gemiddeld werden de deelnemers gevolgd gedurende 6,7 jaar en ontwikkelde 94 van hen hartfalen.

Een aantal jaar geleden werd het behandel effect van darapladib, een selectieve Lp-

PLA2-remmer, geëvalueerd in een gerandomiseerde en gecontroleerde studie bij patiënten met stabiel coronairlijden.[9] Darapladib verminderde de activiteit van Lp-PLA2 in patiënten, maar er was geen significante daling in cardiovasculaire eindpunten door het gebruik van darapladib. Wel was ook in deze studie de mate van Lp-PLA2 activiteit geassocieerd met cardiovasculaire eindpunten en ziekenhuisopnames voor hartfalen. Toekomstig onderzoek moet beoordelen wat het behandeleffect is van Lp-PLA2-remmers in een hartfalen populatie. Voor nu lijkt Lp-PLA2 activiteit een biomarker die gebruikt kan worden voor prognostische doeleinden.[10,11]

Naast het feit dat hartfalen veel voorkomt, is het ook een complexe en progressieve ziekte. Er zijn verschillende pathofysiologische mechanismen die een rol spelen en elkaar beïnvloeden.[3] Het beloop van hartfalen is dan ook dynamisch en de onderliggende oorzaak is divers. Van oudsher werd hartfalen gezien als een verminderde knijpkracht van het hart en daarbij verwijding van de linker hartkamer. In het afgelopen decennium is echter duidelijk geworden dat een aanzienlijk deel van de patiënten met het hartfalen syndroom een normale of licht verminderde knijpkracht van het hart heeft. Sommige studies laten zien dat in 50% van de hartfalen patiënten er sprake is van een normale knijpkracht van het hart.[12] In de ESC-richtlijnen van 2016 voor de diagnose en behandeling van acuut en chronisch hartfalen, zijn drie verschillende soorten hartfalen onderscheiden, gebaseerd op de knijpkracht van het hart.[13] Deze drie typen hartfalen zijn hartfalen met een gereduceerde knijpkracht (HFrEF), hartfalen met een licht verminderde knijpkracht (HFmrEF) en hartfalen met een behouden knijpkracht (HFpEF). Hoewel het niet geheel duidelijk is of deze typen hartfalen daadwerkelijk verschillende syndromen vertegenwoordigen of dat ze deel uitmaken van één hartfalen spectrum, is het wel duidelijk dat de demografie, de aanwezigheid van comorbiditeiten en behandel respons verschillend is.[12] De gemiddelde biomarker waarden verschillen tussen patiënten met HFrEF en HFpEF.[14]

We hebben een systematische review geschreven, met als onderwerp biomarkers bij hartfalen patiënten met een behouden knijpkracht van het hart (HFpEF) **(hoofdstuk 8)**. We hebben vastgesteld dat verschillende biomarkers zoals GDF-15, cystatine C, resistine en galectine-3, veelbelovend zijn als diagnostisch hulpmiddel. Andere biomarkers, waaronder TNF-α, IL-6, PINP, PIIINP, osteopontin en cystatine C, lijken een prognostische waarde te hebben bij deze hartfalen patiënten.

Kwaliteit van leven bij patiënten met hartfalen

In **deel II** van dit proefschrift, werd het TRIUMPH-cohort gebruikt voor het evalueren van kwaliteit van leven bij patiënten met hartfalen. Kwaliteit van leven werd onderzocht in

combinatie met de aanwezigheid van comorbiditeiten, ernst van hartfalen symptomen en klachten van depressie. Er is geen gouden standaard voor het meten van de kwaliteit van leven.[15] Om hiervoor te corrigeren, zijn er vier verschillende vragenlijsten afgenomen aan het begin van de studie, bij ontslag van de initiële hartfalen opname, en aan het einde van de studie als patiënten terug kwamen op de polikliniek. Kwaliteit van leven werd beoordeeld met behulp van een specifieke hartfalen vragenlijst, de Kansas City Cardiomyopathy Questionnaire (KCCQ), en een algemene vragenlijst, de EuroQol 5 Dimensions (EQ-5D). Bovendien werden angst en depressieve klachten gemeten met de Hospital Angst and Depression Scale (HADS) en uiteindelijk werd de aanwezigheid van hartfalen symptomen geëvalueerd in een aparte vragenlijst.

Hoofdstuk 9 toont aan dat hartfalen patiënten vaak last hebben van hartfalen symptomen, wat in lijn is met eerder onderzoek. We weten dat de symptoomlast vaak wordt onderschat en daarom onvoldoende behandeld.[16] Voorlichting aan patiënten en behandelaars verdient dan ook de aandacht. Daarnaast hebben we vastgesteld dat de symptoomlast bij patiënten met depressieve klachten toegenomen is ten opzichte van patiënten zonder depressieve klachten. Het is dan ook belangrijk om specifiek bij hartfalen patiënten aandacht te hebben voor depressieve klachten en dit mee te nemen in de behandeling.[17]

In **hoofdstuk 10** hebben we gekeken naar de relatie tussen comorbiditeiten en kwaliteit van leven in hartfalen patiënten en de invloed van bepaalde determinanten op de kwaliteit van leven. In het gehele TRIUMPH-cohort hadden hartfalen patiënten zonder comorbiditeit (eerdere CVA, chronische nierfunctie stoornissen, diabetes mellitus en COPD) een betere kwaliteit van leven in vergelijking met patiënten met comorbiditeit. Dit ondersteunt de opvatting dat om kwaliteit van leven te verbeteren van hartfalen patiënten, er ook aandacht moet zijn voor comorbiditeiten.[18] Daarnaast hebben we onderzocht of bepaalde determinanten van invloed zijn op de kwaliteit van leven in patiënten met en zonder comorbiditeiten. De determinanten die gerelateerd zijn aan kwaliteit van leven in het TRIUMPH-cohort (geslacht, hartfalen in de voorgeschiedenis, lichaamsgewicht index, NT-proBNP waarde bij opname, systolische bloeddruk bij ontslag en de aanwezigheid van een depressie) zijn in lijn met eerdere studies, echter opvallend is dat deze determinanten alleen belangrijk zijn in de subgroep van patiënten met een comorbiditeit.[19]

Er is nog veel onderzoek nodig om vast te stellen hoe de herkenning van symptomen en de behandeling van deze symptomen, depressie en comorbiditeiten bij hartfalen patiënten kan worden verbeterd. Voor veel patiënten is het verbeteren van de kwaliteit

van leven op zijn minst zo belangrijk als het verlengen van het leven.[20,21] Hartfalen symptomen, depressie en comorbiditeiten zijn daarom belangrijke speerpunten in een goede behandeling van patiënten met hartfalen.

RAAS-remmers bij patiënten met hypertensie

In **deel III** van dit proefschrift, **hoofdstuk 11,** hebben we een meta-analyse uitgevoerd met als onderzoeksvraag of de behandeling van patiënten met hypertensie met een renine-angiotensine-aldosteronsysteem (RAAS)-remmer er voor zorgt dat mensen minder snel komen te overlijden. Gegevens van bijna 160.000 patiënten zijn geanalyseerd en we toonden aan dat hypertensie patiënten die behandeld werden met RAAS-remmers minder vaak overleden vergeleken met hypertensie patiënten die niet behandeld werden met RAAS-remmers. De patiënten zijn gemiddeld 4 jaar vervolgd en gedurende deze periode was er een afname in overlijden van 5%. De studies die meegenomen zijn in deze meta-analyse zijn over het algemeen kwalitatief goed opgezet en alle patiënten werden gerandomiseerd. In de verschillende studies werd een diversiteit aan hypertensie patiënten onderzocht, daarnaast werden de patiënten adequaat behandeld voor bijkomende risicofactoren, hierdoor is de uitkomst van deze meta-analyse goed te generaliseren naar de algemene hypertensie populatie in de dagelijkse klinische praktijk.

Op dit moment zijn de belangrijkste farmacologische middelen die het RAAS blokkeren, de ACE-remmers en AT1-receptorblokkers (ARB). Hoewel ACE-remmers en ARB's beide RAAS-remmers zijn, hebben ze verschillende werkingsmechanismen.[22] We hebben een post-hoc analyse uitgevoerd, waarbij we stratificeerden voor deze twee groepen medicamenten. We zagen dat de ACE-remmers de kans op overlijden gedurende 4 jaar met 10% verlaagden ten opzichte van de patiënten die behandeld werden zonder een RAAS-remmer, terwijl de ARB's een neutraal behandeleffect toonden ten opzichte van een behandeling zonder een RAAS-remmer. Benadrukt moet worden dat we deze meta-analyse niet hebben ontworpen om deze twee groepen medicamenten met elkaar te vergelijken en gezien dit geen rechtstreeks vergelijking is moeten we voorzichtig zijn met het trekken van conclusies.

Aanvullend hebben we in **hoofdstuk 12** een analyse gedaan waarin we gekeken hebben naar het aantal patiënten wat behandeld moest worden om één overlijden te voorkomen, een 'Number Needed to Treat' (NNT) analyse. Dit hebben we gedaan om inzicht te krijgen in het absolute behandel effect van RAAS-remmers in plaats van enkel het relatieve risico.[23, 24] Ons onderzoek toonde dat we 113 hypertensie patiënten gedurende 4 jaar (2,5-97,5e percentiel, 85 tot 168) met een RAAS-remmer moeten behandelen, in plaats van placebo of een andere bloeddrukverlager, om één overlijden te voorkomen.

We hebben verschillende eindpunten onderzocht (sterfte, cardiovasculaire sterfte, hartinfarct en beroerte) en gekeken naar de effectiviteit van ACE-remmers en ARB's afzonderlijk. ACE-remmers verlagen de kans op sterfte (NNT 67), cardiovasculaire sterfte (NNT 116) en een hartinfarct (NNT 80). De overeenkomstige NNT gedurende 4 jaar door behandeling met een ARB was hoger en niet significant (NNT 335 voor sterfte, NNT 409 voor cardiovasculaire sterfte en NNT 338 voor myocardinfarct). Het behandeleffect van ACE-remmers en ARB's voor beroerte was vergelijkbaar.

De meest effectieve behandelingen hebben de laagste NNT. De klinische consequentie met betrekking tot de NNT hangt af van verschillende parameters, zoals effectiviteit, duur van de behandeling, kosten en bijwerkingen van de behandeling naast het absolute risico en de prevalentie van de ziekte in de algemene bevolking.[23,25] Hypertensie komt veel voor in de algemene bevolking[26] en rekening houdend met het relatief lage absolute sterfterisico, lijkt het behandelen van 113 patiënten gedurende 4 jaar om één overlijden te voorkomen relevant. Bovendien is er naast de afname van de sterfte ook nog sprake van een afname van het aantal hartinfarcten en beroertes. Deze kennis zal artsen moeten aanmoedigen om patiënten met hypertensie te behandelen met een RAAS-remmer.

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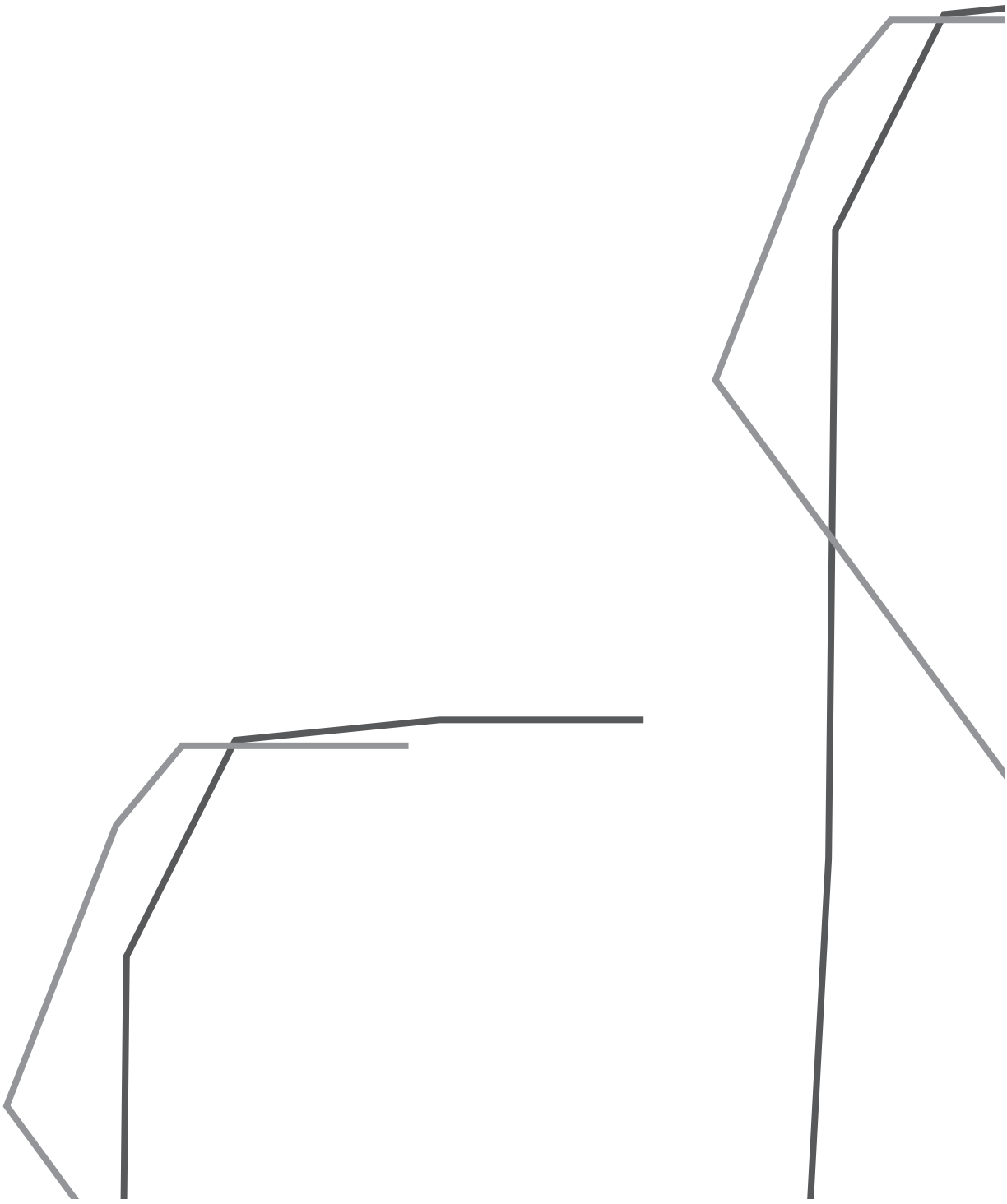

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Submitted

About the Author

Laura Charlotte van Vark was born on July 13th 1982, in Zwijndrecht, the Netherlands. After finishing secondary school in 2000 (VWO, Interconfessioneel Makeblijde College, Rijswijk), she studied A-levels Mathematics and Physics at the Chichester College of Arts, Science and Technology, United Kingdom. In 2001 she started her medical training at the Erasmus University Rotterdam. In 2003, she was selected to participate in the Master degree programme for excellent medical students at the Erasmus MC to coincide with her medical training. As part of this program, she studied (cardiovascular) epidemiology at Cambridge University, United Kingdom and at Johns Hopkins Bloomberg School of Public Health, Baltimore, United States of America. After participating in research in the Rotterdam Study at the department of Epidemiology & Biostatistics (supervision: Prof. Dr. J.C.M. Witteman), she completed the Master of Science program in Clinical Epidemiology at the Netherlands Institute for Health Sciences in 2005 (supervision: Prof. Dr. A. Hofman). During her internships, she spent a research internship abroad at the Shoklo Malaria Research Unit, Mae Sot, Thailand. In 2008, she graduated from medical school and began to work as a cardiology resident at the Erasmus MC in Rotterdam. In 2009 she initiated her PhD concerning a multi-center study on biomarkers in heart failure patients (TRIUMPH project), under the supervision of Prof. dr, ir. H. Boersma. In 2011 she started working as a resident in the internal medicine department at the Albert Schweitzer hospital in Dordrecht (supervision: Dr. E.F.H. van Bommel) as part of her cardiology training. She returned to the Erasmus MC to resume her PhD project in 2013 and continued her cardiology training in 2015 as a resident in the cardiology department at the Albert Schweitzer hospital in Dordrecht (supervision: Dr. M.J.M. Kofflard and Dr. E.J. van den Bos) and Erasmus MC in Rotterdam (supervision: Dr. F.J. ten Cate and Dr. T.W. Galema).

The 9th of July 2020 will be a special day for her, as she will finish her cardiology training and defend her thesis ‘Biomarkers to improve prognostication in heart failure’ on the same day. Afterwards she will start her work as a cardiologist, at Saxenburgh MC, with special interest in heart failure, cardiac MRI and geriatrics.