PROGNOSIS IN HEART FAILURE

PROGNOSIS IN CURRENT HEART FAILURE PATIENTS

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ABSTRACT

Background: Heart failure (HF) constitutes an important growing medical and economic problem with high prevalence and mortality. Prognosis assessment remains a challenge because of the dynamic nature of HF and the existence of some unexplained variation in outcomes. Our objective was to refine the process of prognostic assessment in current HF patients.

Methods: We conducted a systematic review to identify existing risk predictive models in ambulatory HF patients, a meta-analysis to identify mortality predictors in HF patients treated with an implantable cardioverter defibrillator (ICD), a retrospective cohort study to validate a new model, the HF Meta-Score, derived from the results of the meta-analysis and a cross-sectional and prospective cohort study to evaluate whether circulating progenitor cells (CPCs) are associated with functional capacity and mortality in ambulatory HF patients.

Results: We identified 20 risk predictive models in ambulatory HF patients; only five were externally validated showing limited discrimination and calibration. The two most validated models were derived from HF cohorts from the 1990s and reported limited performance in ICD patients. In a meta-analysis, we identified that age, baseline renal function, history of symptomatic HF, chronic obstructive pulmonary disease, diabetes, peripheral vascular disease, left ventricular ejection fraction, NYHA class, atrial fibrillation, wide QRS and the occurrence of appropriate or inappropriate ICD shocks were independent mortality predictors. Some of these predictors were omitted in

previously identified models. From the results of the meta-analysis, we developed the HF Meta-Score that showed better performance than an existing model. We observed that CPCs were independently associated with functional capacity and outcomes in ambulatory HF patients.

Conclusions: These results open many pathways to further refine the prognostic assessment in ambulatory HF patients. The HF Meta-Score is a promising score. The clinical utility of the HF Meta-Score and of the incorporation of new predictive factors, such as CPCs, needs to be tested.

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LIST OF ABBREVATIONS

ACEI	angiotensin converter enzyme inhibitor
AIC	Akaike information criterion
ARB	angiotensin receptor blocker
ATP	anti-tachycardia pacing
BMI	body mass index
BNP	brain natriuretic peptide
BUN	blood urea nitrogen
CAD	coronary artery disease
CI	confidence interval
CMP	cardiomyopathy
CMR	cardiovascular magnetic resonance
COPD	chronic obstructive pulmonary disease
CPCs	circulating progenitor cells
CPET	cardiopulmonary exercise testing
CPR	c-reactive protein
CRD	chronic renal dysfunction
CRT	cardiac resynchronization therapy
EO-CFU	early outgrowth colony forming units
EPC	endothelial progenitor cells
GFR	glomerular filtration rate
GOF	goodness-of-fit
GRADE	Grading of Recommendations, Assessment, Development and Evaluation
HF	heart failure
HFSS	Heart Failure Survival Score

H-L	Hosmer-Lemeshow
HR	hazard ratio
HTx	heart transplantation
ICD	internal cardiac defibrillator
IQR	inter-quartile range
LR	likelihood
LVEF	left ventricular ejection fraction
MCS	mechanical circulatory support
MI	myocardial infarction
NO	nitric oxide
NRI	net reclassification improvement
NYHA	New York Heart Association
OUES	oxygen uptake efficiency slope
PACE	peripheral vascular disease, age, creatinine, ejection fraction
PBMC	peripheral blood mononuclear cells
PVD	peripheral vascular disease
RCT	randomized control trial
RER	respiratory exchange ratio
ROC	receiver operating Curve
SD	standard deviation
SHFM	Seattle Heart Failure Model
TNF-	tumor necrosis factor-alpha
UNOS	United Organ Network Sharing
VAD	ventricular assist device
VEGFR2	vascular endothelial growth factor receptor 2
VO_2	oxygen consumption

DECLARATION OF ACADEMIC ACHIVEMENT

I was the primary responsible and leader of the research conducted in this thesis. I conceived the research idea and design of all the included projects. I requested Research Ethic Board approval when necessary. I acquired and analyzed the data. I performed all the laboratory measurements of circulating progenitor cells. I performed citation screenings in the systematic reviews related to this thesis. I created all the graphs and tables and interpreted the results. I drafted and prepared the manuscripts for publication. I responded to reviewers' queries during the publication process and reviewed the galley proofs of accepted manuscripts. I applied for funding.

Dr. Gordon Guyatt, Dr. Stephen Walter and Dr. Heather Ross acted as my supervisors.

Other co-authors participated in data collection, citation screenings and data abstraction, and reviewing the manuscripts prepared for publication.

CHAPTER I

INTRODUCTION

Heart failure (HF) constitutes an important growing medical and economic problem. Although reliable statistics are lacking in many countries, in 2010, the prevalence of HF has been estimated as 2.1% of the adult population in the United States and increases with age [1]. Over 26 million people suffer from HF around the world [2-3]. In Canada, based on 2000-2001 data, 1% of the adult general population have congestive HF [4]. Over 600,000 people are newly diagnosed with HF every year in Europe [5] and 670,000 in the United States [3]. The longer life expectancy of the population, better treatment of heart diseases, and increase in risk factors for ischemic heart disease, one of the main HF causes, fuel the growing incidence and prevalence of HF around the world.

The prognosis associated with HF is poor. Overall survival in HF patients is inferior to most cancer patients, with a 50% mortality after 4 years from diagnosis [6]. In 2009, 1 in 9 death certificates (274,601 deaths) in the United States mentioned HF as cause of death [1]. Based on these statistics, 1 in 5 adults over 40 years will have HF in their lifetime; and 1 of 5 HF patients will die within a year of diagnosis.

The economic cost of HF is estimated to be in billions of dollars per year in the United States. The need for repeated hospitalization is the most powerful contributing factor to direct costs associated with the disease. Estimated total costs for HF in the United States were $31 billion in 2012 and are anticipated to increase to $70 billion in 2030 assuming that all costs are related to treating HF and not to comorbidities.

The statistics and costs associated with HF are overwhelming. Strategies to decrease the clinical and economic burden of HF are directed to better control of cardiovascular risk factors through education of the general population. However, once HF is established, the best strategy relies on aggressive treatment, adequate prognostic assessment to optimize patient management, thereby increasing survival and ensuring adequate use of the limited resources.

All the work performed in this thesis is related to improving prognostic assessment in HF patients.

Prognosis assessment in HF patients

Typically HF is progressive. After diagnose and initiation of treatment, patients usually experience clinical stability of variable duration. Eventually patients will have a gradual decline characterized by refractory symptoms, ultimately leading to a need for advanced therapy including transplant and mechanical support, or death [8].

Medical decisions throughout this course are based on serial prognostic assessment. Prognostic assessment of patients with HF is complex due to the increasing proportion of elderly HF patients, multiple co-morbidities, different patterns of disease

progression, continuous improvement in patient management and the development of new therapeutic options. These factors, and their interactions, result in challenges in the prediction of outcomes and consequently the decision-making process.

In the past decade, there has been a growing interest in developing scores to estimate prognosis. The main reason for the development and use of predictive scores is that no single prognostic factor reliably predicts outcome. These risk assessment scores have been derived from different HF populations. They use a variety of variables to predict prognosis and few of them have demonstrated reasonable accuracy in validation studies.

Prognosis assessment remains a challenge because of the dynamic nature of the disease process and the existence of some unexplained variation in outcomes. The management of HF patients is under steady refinement. One of the most significant recent changes in the management of HF patients is the increased use of internal cardioverter defibrillator (ICD). ICD therapy is indicated to prevent sudden cardiac death in patients with heart disease and currently it constitutes part of the standard medical management of patients with HF [9-11]. Based on results from randomized controlled trials, current ICD indications are as a secondary prevention in patients with confirmed or highly suspicious life-threatening tachyarrhythmia; or as primary prevention in HF patients on optimal medical treatment, with ischemic or non-ischemic cardiomyopathy, mildly to moderately symptomatic with low left ventricular ejection fraction (LVEF $\leq$35%). These indications have led to a substantial increase (100%) in the utilization of ICD between 2003 and 2005 in North America and Europe [12]. Prognostic factors and survival scores that were

identified and developed before the increased use of ICDs may act differently in current ICD treated HF patients. Ignoring this fact when extrapolating the available evidence to current HF patients may compromise adequate prognosis assessment.

Adequate assessment of prognosis relies on our experience and knowledge. The pathophysiology of HF is not yet completely understood. This inevitable uncertainty limits the performance of existing predictive models. Efforts to reduce this uncertainty through the identification of new prognostic factors and their additional predictive value is worthwhile and may refine the process of prognosis assessment. An interesting new prognostic factor that may play an important role in the pathophysiology of cardiovascular diseases is a group of cells called circulating progenitor cells (CPC).

Circulating progenitor cells are blood circulating cells activated in response to ischemic insults and which regulate adult vasculogenesis and endothelial function. In the 1990s, it was commonly accepted that postnatal angiogenesis occurred exclusively through the local outgrowth of pre-existing vessels by means of expansion of mature endothelial cells in response to angiogenic growth factors. However, an enriched population of CD34+ cells, isolated from human peripheral blood, were subsequently shown to differentiate into endothelial cells in vitro and, in mice, were incorporated into areas of angiogenesis after ischemia [13]. These findings provided the first direct evidence about the existence of adult neovascularization and led to the possibility of novel therapeutic targets for tissue repair after ischemic injury and potential new prognostic factors.

Inflammatory activation, endothelial dysfunction and endothelial damage are important in the pathogenesis of HF, contributing to cardiac remodelling and peripheral vascular disturbances [14]. The presence and degree of endothelial dysfunction has been associated with disease progression and outcomes in patients with HF [15-17]. In the clinical arena, studies analyzing the association between CPCs and morbidity and mortality in HF patients are characterized by controversial results. Circulating progenitor cells represent an innovative marker with potential prognostic and therapeutic value. Clinical studies focusing on the prognostic role of CPC in HF may help to deepen our understanding, and potentially enhance prognostic assessment.

Therefore, the objective of this thesis was to refine the process of prognosis assessment in HF patients by:

1. **Conducting a systematic review to identify studies evaluating the use of risk prediction models for mortality in ambulatory HF patients, describe their performance and their clinical applicability.** This objective is addressed in the following paper:
    a. Alba AC, Agoritsas T, Jankowski M, Courvoisier DS, Walter S, Guyatt GH, Ross HJ. Risk prediction models for mortality in ambulatory heart failure patients: A systematic review. Circulation Heart Failure 2013; 6(5):881-889.

2. **Conducting a systematic review and meta-analysis to identify factors associated with mortality in ICD patients and to assess the magnitude of these associations.** This objective is addressed in the following paper:
    a. Alba AC, Braga J, Gewarges M, Walter S, Guyatt G and Ross HJ. Predictors of mortality in patients with an implantable cardiac defibrillator: A systematic review and meta-analysis. Can J Cardiol 2013; in press.
3. **Constructing a predictive model from the results of the meta-analysis conducted in objective 2. The rationale was based on enhanced model generalizability and performance by incorporating many important predictors of mortality described in ICD patients.** This objective is addressed in the following paper:
    a. Alba AC, Walter S, Guyatt G and Ross HJ. Predictors of mortality in patients with an implantable cardiac defibrillator: A systematic review and meta-analysis. Submitted for publication to Circulation in October 2013.
4. **Exploring whether circulating progenitor cells constitute an independent predictor of functional capacity and mortality in ambulatory HF patients.** This objective is addressed in the following papers:
    a. Alba AC, Delgado DH, Rao V, Walter S, Guyatt G and Ross HJ. Are endothelial progenitor cells a prognostic factor in patients with heart failure? Expert Rev Cardiovasc Ther 2012; 10:167-175.

b. Alba AC, Lalonde S, Rao V, Walter S, Guyatt G and Ross HJ. Endothelial progenitor cells and functional capacity in heart failure patients. Can J Cardiol 2013; 29(6):664-671.

c. Alba AC, Lalonde S, Rao V, Walter S, Guyatt G and Ross HJ. Circulating Progenitor cells and functional capacity and mortality in heart failure patients: A longitudinal study. Can J Cardiol 2013; in press.

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CHAPTER II

The work described in this chapter has been published in Circulation Heart Failure.

(Alba et al. Circulation Heart Failure 2013; 2013 6(5):881-889.)

TITLE: Risk prediction models for mortality in ambulatory heart failure patients: A systematic review

Short title: Prediction models in heart failure

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ABSTRACT

Background: Optimal management of heart failure (HF) requires accurate assessment of prognosis. Many prognostic models are available. Our objective was to identify studies evaluating the use of risk prediction models for mortality in ambulatory HF patients, describe their performance and clinical applicability.

Methods and Results: We searched for studies in MEDLINE, EMBASE and CINAHL in May 2012. Two reviewers selected citations including HF patients and reporting on model performance in derivation and/or validation cohorts. We abstracted data related to population, outcomes, study quality and model discrimination and calibration. Of the 9952 reviewed, we included 34 studies testing 20 models. Only 5 models were validated in independent cohorts: the Heart Failure Survival Score (HFSS), the Seattle Heart Failure Model (SHFM), the PACE risk score, a model by Frankenstein et al and the SHOCKED predictors. HFSS was validated in 8 cohorts (2240 patients) showing poor to modest discrimination (c-statistic 0.56-0.79), being lower in more recent cohorts. SHFM was validated in 14 cohorts (16057 patients) describing poor to acceptable discrimination (0.63-0.81), remaining relatively stable over time. Both models reported adequate calibration, though overestimating survival in specific populations. The other three models were validated in one cohort each, reporting poor to modest discrimination (0.66-0.74). Among the remaining 15 models, six were validated by bootstrapping (c-statistic 0.74-0.85); the rest were not validated.

Conclusions: Externally validated HF models showed inconsistent performance. The HFSS and SHFM demonstrated modest discrimination and questionable calibration. A

new model derived from contemporary patient cohorts may be required for improved prognostic performance.

Key words: heart failure, survival, prognosis, prediction models.

INTRODUCTION

Heart failure (HF) is a frequent health problem with high morbidity and mortality, increasing prevalence, and escalating healthcare costs [1,2]. Older patient age, multiple co-morbidities, and different patterns of disease progression create important challenges in patient management. Because the impact of these factors, and their interactions, remain incompletely understood, predicting patients' clinical course is difficult.

Accurate estimation of prognosis is important for many reasons. Patients are concerned about their probability of future events. Physicians may use prognosis estimates to decide the appropriate type and timing of additional tests or therapies, including heart transplantation and mechanical circulatory support. Accurate prognostic assessment may prevent delays in appropriate treatment of high risk patients or overtreatment of low risk patients. Knowledge of prognosis also facilitates research, for instance in the design of randomized trials and the exploration of sub-group effects.

To be usefully applied, prognostic models must be accurate and generalizable. Models may be inaccurate due to omission of important predictors, derivation from unrepresentative cohorts, overfitting or violations of model assumptions.

In the past three decades, investigators have developed many models to predict adverse outcomes in HF patients [3,4]. Clinicians and researchers wishing to use prognostic models would benefit from knowledge of their characteristics and performance. We therefore conducted a systematic review to identify studies evaluating the use of risk prediction models for mortality in ambulatory HF patients, describe their performance and their clinical applicability.

METHODS

Data sources and searches: In May 2012, with the assistance of an experienced research librarian, we conducted a systematic search of electronic databases, including Medline, Embase and CINAHL. We used several related terms: (“internal cardiac defibrillator) AND (“heart” OR “cardiac”) AND (“mortality” OR “survival”) AND ("multivariate analysis" OR "regression analysis” OR “risk factor” OR “prediction” OR “prognostic factor”). The full search strategy is outlined in Appendix A (Supplemental methods). We identified additional studies by searching bibliographic references of included publications.

Study selection: Eligible articles enrolled adults (>19 years) who were ambulatory HF patients; used multivariable analysis (at least two independent variables) to predict mortality or a composite outcome including mortality; reported more than 30 deaths; reported results as a score, a prediction rule or a set of regression coefficients sufficient to make predictions for individual patients; and reported a measure of discrimination or calibration. We also included studies evaluating the performance of an existing score in a different population to the one from which it was developed, and reported model discrimination and/or calibration. There were no restrictions on study design, left ventricular function (LVEF), language or date of publication. We excluded studies that enrolled patients during hospital admission, or duplicate studies providing no new relevant data.

Two reviewers independently screened titles and abstracts and then evaluated full-text versions of all articles deemed potentially relevant by either reviewer. During full

text screening, in cases of disagreement, consensus was reached through discussion. If consensus could not be reached, a third reviewer resolved the issue. Agreement between reviewers was assessed using weighted kappa (0.92). Appendix B (Supplemental methods) shows the eligibility form.

Data extraction: From each study, we abstracted data related to eligibility criteria, data source, time frame of recruitment and characteristics of the population, including age, sex, ischemic cardiomyopathy, left ventricular ejection fraction, use of β-blockers and internal cardiac defibrillator (ICD), definition and number of events. We also identified variables included in the prediction models.

Assessment of study quality and model adequacy and performance: The assessment of study quality and model performance was based on what authors reported in their published articles. The selection of items for the assessment of study quality and model adequacy and performance was based on the criteria proposed by Concato et al [5] and Moons et al [6]. Items included whether patient selection was consecutive, whether the data was collected prospectively, whether the percentage of missing data was small (<5%) and was correctly managed (i.e. using data imputation), whether patients lost to follow up were infrequent (<1%), and whether predictors were coded clearly.

In order to assess model adequacy we abstracted information related to model derivation, including selection of the variables, coding, linearity of the response for continuous variables, overfitting [7] and model assumptions. In order to assess model performance, we abstracted data related to discrimination and calibration. Discrimination expresses the extent to which the model is capable of differentiating patients who had

events from those who did not. It is commonly assessed using the c-statistic, which is equivalent to the area under the Receiver Operating Characteristic (ROC) curve [8]. Model discrimination was deemed as poor if the c-statistic was between 0.50 - 0.70, modest between 0.70 - 0.80 and acceptable > 0.80 [9]. In order to assess how changes in HF treatment might modify model performance, we evaluated the impact of β-blockers, use of ICD and study recruitment date on model discrimination graphically including models tested in more than one external cohort.

The calibration and goodness-of-fit of a model involves investigating how close the values predicted by the model are to the observed values. We identified the method used to assess model calibration (i.e., Hosmer-Lemeshow (H-L) test or deviance, Cox-Snell analysis, correlation between observed vs. predicted events) and estimate of performance.

Supplemental Table 1 explains the criteria used to assess model adequacy and performance in more detail. Items that were not relevant (e.g. in studies validating a pre-existing model) were coded as "non-applicable".

Data synthesis: We summarized the data, focusing on the characteristics of the population from whence models were derived and validated, and the models' performance. We report findings in two sections according to external validation (models that were or were not validated in an independent cohort were summarized separately).

RESULTS

After duplicate citations were removed, we screened 6917 citations and ultimately selected 32 studies evaluating 20 prediction models (Figure 1). Only 5 of these models [10-14] were validated in an independent cohort. Among the remaining 15 models, six were internally validated by bootstrap; the remaining models were not validated.

Prediction models validated in an independent cohort

The Heart Failure Survival Score (HFSS) [10], the Seattle Heart Failure Model (SHFM) [11], the model proposed by Frankenstein et al [12], the PACE risk score [13] and the SHOCKED predictors [14] were validated in a different cohort of HF patients from the model derivation cohort. Supplemental Table 2 and Table 3, and Table 1 summarize the characteristics of studies included, the assessment of study quality and model characteristics, respectively.

Heart Failure Survival Score: The HFSS includes seven variables to predict a composite outcome of death, urgent (UNOS status 1) heart transplantation (HTx) and ventricular assist device (VAD) implantation. Two predictors are binary: ischemic cardiomyopathy and presence of intra-ventricular conduction delay (QRS >120 milliseconds); and 5 are continuous: LVEF, resting heart rate, mean blood pressure, peak oxygen consumption (VO_2) and serum sodium. Scores are then divided in 3 categories: high-risk, medium-risk and low-risk according to pre-specified thresholds [10]. The HFSS was derived from a single centre cohort including 268 HF patients and has been

validated in 8 independent single-centre cohorts including a total of 2240 HF patients [10,14-19].

The validation cohorts involve a broad variety of patient populations (Supplemental Table 2), with a mean age from 51 to 70 years, mostly males (65% - 82%) with a mean LVEF between 20% and 30%. In 3 cohorts, the frequency of use of β-blockers was lower than 30% and in the remaining 4 cohorts was 64% to 80%. In 4 studies reporting ICD status, the frequency of ICD use was 11%, 19%, 49% and 78%.

Model discrimination (assessed by the c-statistic at 1 year) in validation cohorts ranged from poor to modest (0.56 to 0.79), being modest (between 0.70 and 0.79) in six (75%) of the eight validation cohorts. As shown in figure 2, model discrimination was worse in cohorts with more frequent use of β-blockers or ICDs, and in more recent studies. Discrimination was poor (c-statistic < 0.70) in validation cohorts in which the rate of ICD use was higher than 40%, studies with a contemporary recruitment date and in 3 of 4 cohorts in which the use β-blockers was higher than 60%. The study by Zugck et al [15] reported a substantially higher discrimination (c-statistic= 0.84 at 1 year) when peak VO_2 was replaced by the 6-minute walk test (6'WT). However, this HFSS variant has not been further validated. Only one study [18] assessed HFSS model calibration and reported that the model overestimated event-free survival by approximately 20% in low risk patients.

Seattle Heart Failure Model: The SHFM includes ten continuous variables (age, LVEF, NYHA class, systolic blood pressure, diuretic dose adjusted by weight, lymphocyte count, hemoglobin, serum sodium, total cholesterol and uric acid) and ten

categorical variables (sex, ischemic cardiomyopathy, QRS > 120 milliseconds, use of β-blockers, angiotensin converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARB), Potassium(K)-sparing diuretic, statins and allopurinol, and ICD/cardiac resynchronization therapy (CRT) status) in an equation that provides a continuous risk score for each patient, and which can be expressed as predicted mean life expectancy or event-free survival at 1, 2 and 5 years [11]. This model was developed to predict a composite outcome of death, urgent HTx and VAD in 1125 HF patients enrolled in the randomized controlled trial (RCT) PRAISE-1 (Prospective Randomized Amlodipine Survival Evaluation). The SHFM has been validated in 14 independent cohorts including 16057 HF patients (4 cohorts including 8983 HF patients were selected from RCTs (Supplemental Table 2)) [11,18,22-28]. The validation cohorts involve diverse populations with a mean age from 52 to 77 years, a higher proportion of males (61% - 82%) and mean LVEF between 17% and 45%. In 4 cohorts, the used of β-blockers was 20% to 35% and in the remaining cohorts was higher than 60% (maximum of 92%). In 10 studies reporting ICD status, the use of ICD was lower than 25% in 5 cohorts and higher than 65% in 3 cohorts.

Model discrimination varied from poor to acceptable (0.63 to 0.81), being at least modest (>0.70) in 7 (50%) cohorts of the 14 validation cohorts. There was a slight trend toward poorer discrimination in cohorts with higher use of ICD devices but was only weakly related to β-blocker use and recruitment date (Figure 2). Some studies [18,22,25] have analyzed variations of the SHFM including other predictors, such as renal function, diabetes, peak VO_2 and BNP and reported that discrimination did not improve

significantly. May et al [22], however, reported that discrimination was significantly improved from 0.72 to 0.78 when BNP was added to the model. Model calibration was evaluated in most of the cohorts (Table 1), and showed a high correlation (r-coefficient >0.97) between observed and predicted survival. In 3 cohorts, calibration was assessed graphically by comparing observed and predicted event-free survival [17,22,24]; the model overestimated event-free survival by around 2% at 1 year and 10% at 5 years, more significantly in African-American and ICD/CRT patients [22]. The study by Kalogeropoulos et al [24] reported inadequate model goodness-of-fit as assessed by the H-L test.

Frankenstein et al's model: This model includes 2 binary variables: BNP and 6'WT with different cut-offs depending on sex and use of β-blockers [12]. Patients can then be categorized in 3 groups (scores 0, 1 or 2). This model was derived from 636 HF patients to predict all-cause mortality and validated in an independent cohort of 676 HF patients (mean age 74 years, 76% male, 63% ischemic CMP, 54% treated with β-blockers). Model discrimination in the validation cohort was poor, varying from 0.66 to 0.68 (Table 1). Model calibration was not reported.

PACE risk score: This model includes 4 binary variables: the presence of peripheral vascular disease, age >70 years, creatinine >2mg/dL and LVEF <20% and provides a continuous risk score for an individual patient from 0 to 5 [13]. This model was derived from 905 secondary and primary prevention ICD patients to predict all-cause mortality and validated in an independent cohort of 1812 ICD-HF patients (mean age of 64 years, 77% male, mean LVEF of 31% and 58% had ischemic CMP (Supplemental

Table 2)). Model discrimination in the validation cohort was poor with a c-statistic at 1 year of 0.69 (Table 1). Model calibration was not reported.

SHOCKED predictors: This model includes 7 binary variables: age >75 years, NYHA class >II, atrial fibrillation, chronic obstructive pulmonary disease, chronic kidney disease, LVEF <20% and diabetes [14]. This score provides a continuous risk score from 0 to 400 and estimates 1-, 2-, 3- and 4- year survival using a nomogram. This model was derived and validated from a cohort of Medicare beneficiaries receiving primary prevention ICD. The validation cohort included 27893 patients (39% of patients were >75 years, 75% male, 31% had LVEF <20% and 63% had ischemic CMP (Supplemental Table 2)). Model discrimination in the validation cohort was modest with a c-statistic at 1 year of 0.74 (Table 1). Overall correlation between observed and predicted survival was high correlation (r-coefficient >0.89). However, model calibration, assessed by H-L test, showed inadequate goodness of fit at 2 and 3 years.

Prediction models not validated in an independent cohort

We identified 15 prediction models that were not validated in an external cohort. Supplemental Tables 4, 5 and 6 summarize the characteristics of studies included, the assessment of study quality and model characteristics, respectively. These models include a wide variety of predictors tested in diverse HF populations. The number of predictors included ranged from 2 to 21. Seven models were derived from patients with reduced LVEF and one in patients with preserved LVEF. The remaining studies included clinically diagnosed HF patients without considering a specific LVEF cut-off as an

inclusion criterion. In 6 studies internally validated by bootstrapping, model discrimination ranged from 0.74 to 0.85. The best discrimination (c-statistic = 0.85) was observed in the DSC index, a model derived from a selective cohort of HF patients undergoing CRT implantation, which included some variables that are not routinely available: one binary variable, postero-lateral scar location evaluated by cardiovascular magnetic resonance (CMR); and 2 continuous variables, tissue synchronization index measured by CMR and serum creatinine. The 5 studies that evaluated model calibration reported adequate performance.

DISCUSSION

In this systematic review, we identified 20 event-free survival prediction models in ambulatory HF patients. Only 25% (5 of 20 models) have been validated in external cohorts and only 2 models, the HFSS and the SHFM, have been validated in more than 2 independent cohorts, mostly reporting modest (0.70-0.80) to poor discrimination (<0.70). Studies using the HFSS more frequently reported modest (>0.70) discrimination than cohorts evaluating the SHFM. However, HFSS performance showed a decline over time whereas the SHFM had a relatively stable performance. Nonetheless, only 2 studies [18,20] have directly compared models within the same population and reported that model discrimination was similar (c-statistic at 1 year of 0.73 and 0.72 [20] and 0.68 and 0.63 [18] for the SHFM and the HFSS, respectively).

Model discrimination represents the model's capacity to differentiate patients who had the event from those who did not. The study by Goda et al [20] reported that

discrimination was significantly higher (from 0.72-0.73 to 0.77 at 1 year) when HFSS and SHFM were used in a combined manner within the same model. May et al [22] reported that the discrimination of the SHFM was significantly improved from 0.72 to 0.78 when BNP was added to the model. As proposed by D'Agostino and Byung-Ho Nam [9], a model with discriminative capacity >0.70 has acceptable discrimination; a discriminative capacity > 0.80 provides strong support to guide medical decision-making. Clearly, HFSS and SHFM have consistently demonstrated that their performance shows only modest discriminative capacity.

One potential reason for suboptimal performance is that the management and treatment of HF patients has changed substantially in the past two decades. These models were derived from cohorts of patients recruited approximately 20 years ago (1986-1991 for the HFSS and 1992-1994 for the SHFM).

As proposed by Moons et al [6] a good model should include variables that are believed to be associated with the outcome of interest. Koelling et al [16] evaluated the association of the seven predictors included in the HFSS model in patients treated with β-blockers and reported that only peak VO_2 and LVEF were factors independently associated with event-free survival. In addition the directions of association of some predictors are opposite in the validation and derivation cohorts. For instance, the HFSS derivation study reported that the hazard ratio for a 1 beat per minute increase in heart rate was 1.02 (95%CI of 1.01-1.04) while in 2 validation cohorts [16,20] including a high proportion of patients treated with β-blockers (>70%), the hazard ratio was 0.98 (95%CI

0.97-1.01). This may partially explain the decline observed in the HFSS discriminatory capacity in more recent validation cohorts.

A similar situation is found with potassium-sparing diuretic use in the SHFM. Levy et al [11] imputed in the calculus of the score a hazard ratio of 0.74 for patients on potassium-sparing diuretics. Goda et al [20] reported a non-significant reverse effect of spironolactone in a contemporary cohort (HR 1.20, 95%CI 0.86-1.48). Importantly this tells us that predictors that were believed or found to be associated with mortality in HF patients 20 years ago may not act similarly in contemporary HF patients. This supports the need to develop and test an up to date prediction model.

Discrimination should not be reported in isolation since a poorly calibrated model can have the same discriminative capacity as a perfectly calibrated model [29]. One limitation of calibration is that assessment techniques do not allow for comparisons between models. In the validation cohorts, both the SHFM and the HFSS showed inadequate calibration due to the model overestimating survival in some groups of patients, including low risk patients, African-Americans and patients with ICD/CRT therapy.

Model ability to predict survival has not been compared to physicians' intuitive predictions. A study by Muntwyler et al [30] showed that primary care physicians overestimated mortality risk in HF patients (1-year observed mortality of 13% vs. physician estimated of 26%); this was more pronounced in stable NYHA class II patients (1-year observed mortality of 6% vs. physician estimated of 18%).

Whether these models may be used to guide or improve clinical practice remains underexplored. Vickers et al [29] has proposed the use of simple decision analytic techniques to compare prediction models in terms of their consequences. These techniques weight true and false positive errors differently, to reflect the impact of decision consequences (i.e. risks associated with HTx or VAD versus risks associated with continuing medical therapy). Such decision analytic techniques may assist in determining whether clinical implementation of prediction models would do more good or more harm relative to current practice (physicians' predictions).

Should use and validation of these models continue? Or should we seek better models? There is no consensus on this issue among commentators. Researchers are pursuing both avenues, validating and supporting the use of the SHFM and HFSS as well as developing new models.

The performance of more recent models developed thus far, however, does not provide evidence that they will perform substantially better than older models. The three externally validated and recently published models [12-14] have demonstrated poor to modest discrimination (between 0.66 - 0.74). Similarly, the 6 models that were validated by bootstrapping showed in general poor to modest discrimination. One of these 6 models provided high discriminatory capacity but it was developed in a selected group of HF patients undergoing CRT implantation and included 2 variables that are not easily measured (myocardial tissue synchronization index and scar location by CMR). The lack of external validation makes it difficult to assess how the performance of the model might

be generalized to other populations, which clearly limits their clinical use. Discrimination estimated on a first sample is often higher than it is on the subsequent samples [31].

Other reasons potentially explaining the suboptimal performance of existing models may pertain to the presence of missing data and variable selection. For example, in cohorts validating the SHFM, the presence of missing data was as high as 100% for percentage of lymphocytes [26] or 65% for uric acid [22]. Whether frequently missing or not easily available variables should be used to develop a score or should be incorporated to standard clinical practice will depend on the strength of the association between the predictors and outcome, the compromised model performance when the variables are not included in the final score and clinical resources. Nonetheless, adequate methods to deal with missing data, such as multiple imputation techniques, are important when evaluating model performance. The exclusion of cases due to missing information may lead to biased results [32].

Variable selection based on statistical significance may lead to suboptimal models. Other techniques, such as stability selection and sub-sampling, have demonstrated to yield more stable models based on a consistent selection of variables decreasing the chances of type I error [33].

As noticed in this review, the performance of predictive models have been traditionally evaluated by the c-statistic, which has been criticized as being insensitive in comparing models and for having limited direct clinical utility. Reclassification tables, reclassification calibration statistic and net reclassification and integrated discrimination improvements are recently developed methods to assess discrimination, calibration and

overall model accuracy. It has been shown that the use of these methods can better guide clinical decision making by offering prognostic information at different risk strata. The use of these techniques is highly recommended during validation of existing or new models.

CONCLUSIONS

Optimal management of HF patients requires accurate assessment of prognosis; making accurate assessment, however, remains challenging. Among 4 externally validated prediction models, the HFSS and SHFM demonstrated modest discriminative capacity and questionable calibration. The clinical impact of medical decision-making guided by the use of these models has not been explored. Given the limitation of current HF models, the development of a new model derived from contemporary patient cohorts is an appealing option. However, the development and reporting of new models should be optimized by adhering to guidelines to guarantee model adequacy. In addition, new models should seek external validation of their generalizability and performance. Evaluation of the clinical impact of decisions based on models relative to current clinical practice would be enormously informative in determining their utility in real world clinical practice.

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Table 1. Model derivation and performance

Study	**Derivation Validation**	**Model/ Variables**	**Selection**	**Linear Gradient**	**Over-fitting**	**Model assumptions**	**Calibration**	**Discrimination (c-statistic)**
Aaronson 1997 [10]	Derivation	**HFSS:** • Heart rate • BP • LVEF • Sodium • Ischemic CMP • IVCD • Peak VO_2	Based on univariable analysis	n.r.	Yes (109 events and 11 variables)	Held	n.r.	At 1 year=0.79 (0.76-0.82)
	Validation in a different cohort	**HFSS**	n/a	n/a	n/a	n.r.	n.r.	At 1 year=0.76 (0.72-0.80) Overall=0.69 (0.62-0.76)
Zugck 2001 [15]	Validation	**HFSS**	n/a	n/a	n/a	n.r.	n.r.	Overall=0.74 (0.70-0.78)
		HFSS replacing peak VO_2 by 6'WT	n/a	n/a	No	n.r.	n.r.	Overall=0.83 (0.79-0.87)

Table 1. Continued

Study	**Derivation Validation**	**Model/ Variables**	**Selection**	**Linear Gradient**	**Over-fitting**	**Model assumptions**	**Calibration**	**Discrimination (c-statistic)**
Koelling 2004 [16]	Validation	**HFSS**	n/a	n/a	n/a	n.r.	n.r.	Not β-blockers: At 1year=0.76 (0.72-0.80) β-blockers: At 1year=0.73 (0.68-0.78)
Parikh 2009 [17]	Validation	**HFSS**	n/a	n/a	n/a	n.r.	n.r.	At 1year=0.76 (0.70-0.83)
Gorodeski 2010 [18]	Validation	**HFSS**	n/a	n/a	n/a	n/a	Tested graphically: overestimated survival in HT candidates and more pronouncedly in non-HT candidates	At 1 year: In HT candidates =0.53 (0.50-0.63) In non-HT candidates = 0.62 (0.55-0.68)

Table 1. Continued

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
Goda 2010 and 2011 [19-21]	Validation	**HFSS**	n/a	n/a	n/a	n.r.	n.r	* At 1 year: Total cohort=0.72 (0.67-0.76) European American (n=417) =0.69 (0.63-0.75) African American (n=125) =0.73 (0.63-0.84) Hispanic American (n=123) =0.76 (0.66-0.85) ICD/CRT patients (n=382) =0.69 (0.63-0.75)

Table 1. Continued

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
Levy 2006 [11]	Derivation	**SHFM:** • Sex • Age • NYHA • Sodium • Uric acid • Cholesterol • Hemoglobin • Lymphocytes • Systolic BP • LVEF • Ischemic CMP • Allopurinol • Diuretic dose • β-blockers • ACEI/ARB • K-sparing diuretic • ICD/CRT	Based on univariable analysis Forward eliminationEffect of some treatments were obtained from previous RCTs or meta-analysis	Checked	No	n.r.	Assessed graphically observed vs. predicted survival by deciles and by correlation (r=0.97)	At 1 year = 0.73 (0.69-0.76)

Table 1. Continued

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
	Validation ELITE2	**SHFM**	n/a	n/a	n/a	n/a	Correlation (r=0.97)	At 1 year=0.67 (0.65-0.71)
	Validation RENAISSANCE	**SHFM**	n/a	n/a	n/a	n/a	Correlation (r=0.97)	At 1 year=0.69 (0.68-0.72)
	Validation Val-HeFT	**SHFM**	n/a	n/a	n/a	n/a	Correlation (r=0.98)	At 1 year=0.81 (0.72-0.90)
	Validation IN-CHF	**SHFM**	n/a	n/a	n/a	n/a	Correlation (r=0.99)	At 1 year=0.75 (0.70-0.80)
	Validation UW	**SHFM**	n/a	n/a	n/a	n/a	Correlation (r=0.99)	At 1 year=0.68 (0.63-0.73)
May 2007 [22]	Validation	**SHFM**	n/a	n/a	n/a	n/a	Correlation (r=0.99)	‡ At 1 year: Total cohort=0.73 (0.71-0.75) Age >75years (n=1339) =0.68 (0.65-0.72) LVEF >40% (n=1634)=0.66 (0.62-0.69) ICD patients (n=693)=0.62 (0.56-0.69)

Table 1. Continued

Study	**Derivation Validation**	**Model/ Variables**	**Selection**	**Linear Gradient**	**Over-fitting**	**Model assumptions**	**Calibration**	**Discrimination (c-statistic)**
Allen 2008 [23]	Validation	**SHFM**	n/a	n/a	n/a	n/a	Assessed graphically. Overestimated survival at 3 years by 8% (72% vs.80%).	At 1 year=0.73
Kalogeoropoulos [24] and Giamouzis [25] 2009	Validation	**SHFM**	n/a	n/a	n/a	n/a	H-L test, inadequate ($p<0.05$). Graphically, adequate after model re-calibration	† At 1 year: Total cohort (n=445)=0.78 ICD/CRT (n=316)=0.78 No ICD/CRT (n=129)=0.79 White (n=223)=0.78 Black (n=198)=0.79

Table 1. Continued

Study	**Derivation Validation**	**Model/ Variables**	**Selection**	**Linear Gradient**	**Over-fitting**	**Model assumptions**	**Calibration**	**Discrimination (c-statistic)**
Levy 2009 [26]	Validation	**SHFM** and effect of IABP and inotropic support added from effect estimates obtained from previous studies	n/a	n/a	n/a	n/a		At 1 year=0.71
Gorodeski 2010 [18]	Validation	**SHFM**	n/a	n/a	n/a	n/a	Tested graphically: overestimated survival in HT candidates and non-HT candidates	§ At 1 year: In HT candidates =0.68 (0.63-0.74) In non-HT candidates = 0.63 (0.57-0.69)

Table 1. Continued

Study	**Derivation Validation**	**Model/ Variables**	**Selection**	**Linear Gradient**	**Over-fitting**	**Model assumptions**	**Calibration**	**Discrimination (c-statistic)**
Goda 2011 [21]	Validation	**SHFM**	n/a	n/a	n/a	n/a	n.r.	* At 1 year=0.73
Perrota [27] 2012	Validation	**SHFM**	n/a	n/a	n/a	n/a	H-L test: p>0.2 at 1, 2 and 3 years	At 1 year=0.70 (0.61-0.79)
Haga [28] 2012	Validation	**SHFM**	n/a	n/a	n/a	n/a	n.r.	Overall=0.68 (0.58-0.78)
Frankenstein 2011 [12]	Derivation	• BNP • 6'WT (different cut-off according to sex and β-blockers)	Based on univariable analysis	n.r.	no	n.r.	n.r.	Overall: Unadjusted=0.76 Sex-adjusted=0.77 BB-adjusted=0.76 Sex-BB-adjusted=0.77
	Validation	**Frankenstein 2011**	n/a	n/a	n/a	n/a	n.r.	Unadjusted=0.66 Sex-adjusted=0.66 β-blockers -adjusted=0.66 Sex- β-blockers -adjust=0.68

Table 1. Continued.

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
Kramer [13] 2012	Derivation	**PACE risk score** • Age >75 years • LVEF <20% • Creatinine • PVD	Based on univariable analysis	n.r.	no	n.r.	n.r.	At 1 year=0.79
	Validation	**PACE risk score**	n/a	n/a	n/a	n/a	n.r.	At 1 year=0.69
Bilchick [14] 2012	Derivation	**SHOCKED** • Age • NYHA • LVEF • COPD • Diabetes • Atrial fibrillation • CKD	Based on clinical importance and statistical analysis	n.r.	no	n.r.	Correlation (r=0.89)	Overall=0.75(0.75-0.76)
	Validation	**SHOCKED predictors**	n/a	n/a	n/a	n/a	Correlation (r=0.89) H-L test: p<0.001	Overall=0.74(0.74-0.75)

* Goda et al reported that c-statistic was significantly higher (c-statistic= 0.77 at 1 year) when HFSS and SHFM were used in a combined manner.

‡ Authors analyzed the additive discriminative value of creatinine, BUN (blood urea nitrogen), diabetes and BNP (c-statistic= 0.74, 0.74, 0.74 and 0.78, respectively).

† Giamouzis et al analyzed the additive of renal function and reported that renal function (BUN) did not significantly change discriminative capacity.

§ Authors analyzed the additive predicted value of BNP, BUN and peak VO_2 and reported non-significant improvement in c-statistic values.

HFSS, Heart Failure Survival Score; BP, blood pressure; LVEF, left ventricular ejection fraction; IVCD, intra-ventricular conduction defect, VO_2, oxygen consumption; 6'WT, six-minute walk test; SHFM, Seattle Heart Failure Model; MI; myocardial infarction; NYHA, New York Heart Association; CMP, cardiomyopathy; ACEI, angiotensin converter enzyme inhibitor; ARB, Angiotensin II receptor blocker; ICD, internal cardiac defibrillator; CRT, cardiac resynchronization therapy; PRAISE, Prospective Randomized Amlodipine Survival Evaluation; ELITE2, Losartan Heart Failure Survival Study; RENAISSANCE, Randomized Etanercept North American Strategy to Study Antagonism of Cytokines; IN-CHF, Italian Congestive Heart Failure Registry; UW, University of Washington HF clinic; IABP, intra-aortic balloon pump; BNP, brain natriuretic peptide; PVD, peripheral vascular disease; COPD, chronic obstructive pulmonary disease; CKD, chronic kidney disease; n.r., not reported; n/a, non applicable.

LEGENDS OF FIGURES

Figure 1. Study selection process. Number of studies during selection.

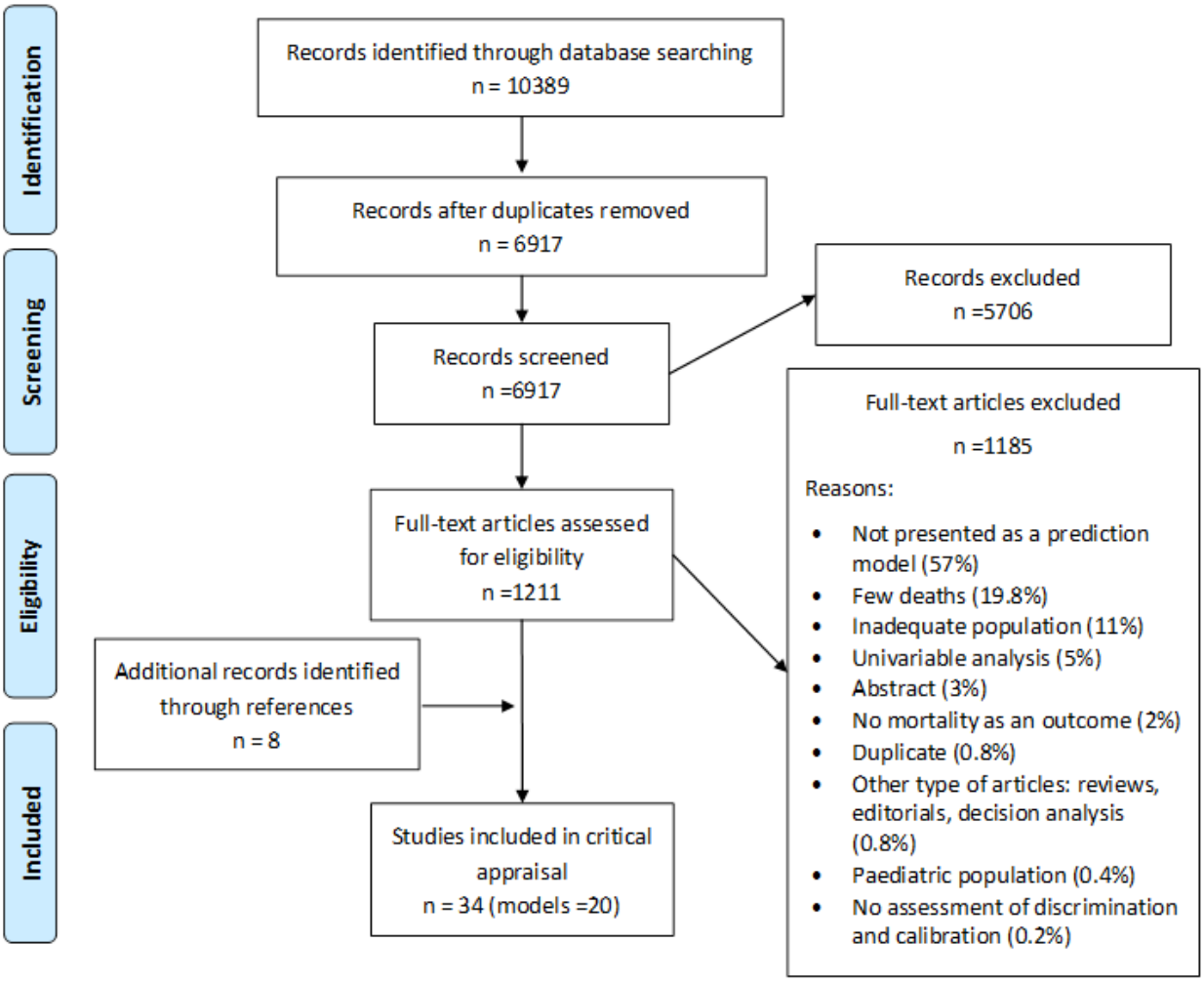
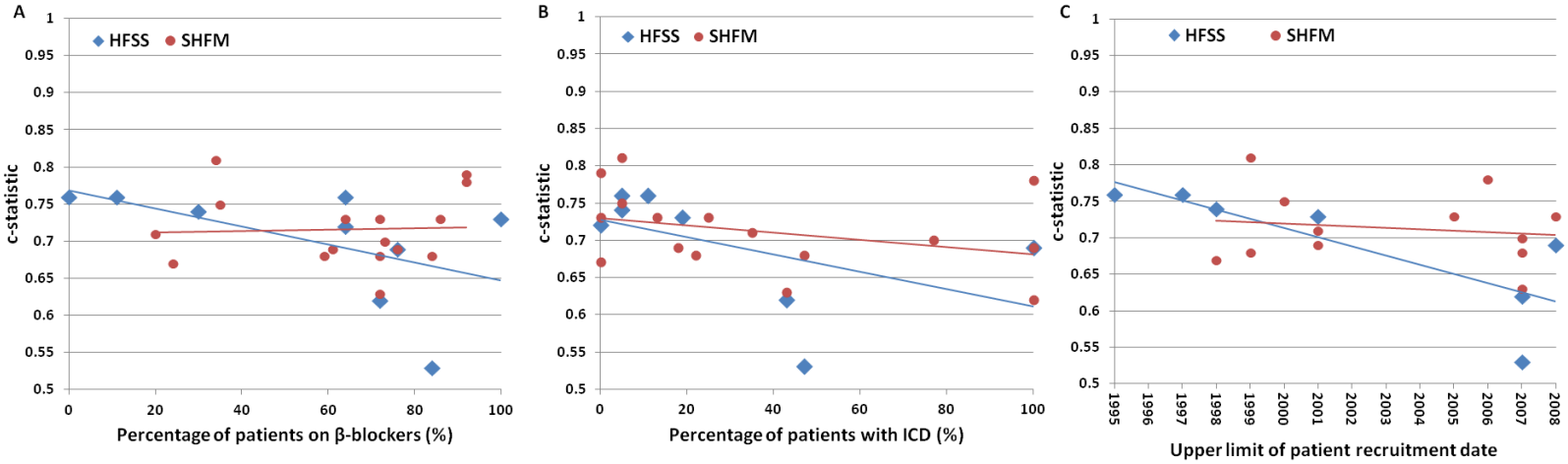


Figure 2. Model discrimination. Model discrimination according to the use of β-blockers (panel A), internal cardiac defibrillator (ICD) (panel B) and study patients recruitment date (panel C).



SUPPLEMENTAL METHODS

Appendix A: Literature Search Results

For: Ana Carolina Alba

Date Completed: 15 May 2012

The databases searched were:

- Ovid MEDLINE
- EMBASE
- CINAHL

RESULTS & STRATEGY USED:

Database: Ovid MEDLINE(R) <1946 to May Week 1 2012>
Search Strategy:
---

1 exp Heart Failure/ (76819)
2 ((heart or cardiac) adj2 failure).mp. (121311)
3 1 or 2 (121859)
4 predict:.mp. (756732)
5 validat:.tw. (180066)
6 scor:.tw. (404761)
7 observ:.mp. (2029286)
8 or/4-7 (3043863)
9 3 and 8 (28134)
10 exp Ambulatory Care/ (42583)
11 Outpatients/ (7351)
12 (ambulatory or stable or chronic or out-patient: or outpatient:).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] (1246085)
13 10 or 11 or 12 (1246085)
14 9 and 13 (8814)
15 (mortality or survival or death).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] (1266793)
16 14 and 15 (3910)
17 statistics as topic/ or exp regression analysis/ (319979)
18 sn.fs. (425839)
19 statistic:.mp. (727873)
20 (logistic adj2 model:).mp. (85018)
21 (Likelihood adj2 function:).mp. (14814)

22 regression:.mp. (356421)
23 exp mathematical concepts/ (626843)
24 algorithm:.mp. (178754)
25 mathematic:.mp. (122305)
26 multivariate analysis/ (66832)
27 exp models, biological/ or exp models, statistical/ or logistic models/ (743997)
28 area under curve/ (21246)
29 or/17-28 (2456770)
30 "review"/ (1691446)
31 risk assessment/ or risk factors/ (590256)
32 evaluation.mp. (1000618)
33 exp Prognosis/ (930163)
34 prognostic factor:.mp. (47548)
35 8 or 31 or 32 or 33 or 34 (4702602)
36 3 and 13 and 15 and 35 (6181)
37 29 and 36 (2602)
38 30 and 36 (1361)
39 37 or 38 (3762)

Database: Embase <1974 to 2012 May 14>
Search Strategy:
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1 exp heart failure/ (244924)
2 ((heart or cardiac) adj2 failure).mp. (207214)
3 1 or 2 (278699)
4 predict:.mp. (983853)
5 validat:.tw. (256546)
6 scor:.tw. (563146)
7 observ:.mp. (2609157)
8 risk assessment/ (285564)
9 risk factor/ (519981)
10 evaluation.mp. (1128376)
11 exp prognosis/ (388902)
12 prognostic factor:.mp. (67942)
13 or/4-12 (5511416)
14 3 and 13 (97265)
15 exp ambulatory care/ (35968)
16 outpatient/ (40332)
17 outpatient care/ (18777)
18 (ambulatory or stable or chronic or out-patient: or outpatient:).mp. (1647754)
19 15 or 16 or 17 or 18 (1647754)

20 14 and 19 (24318)
21 (mortality or survival or death).mp. (1806751)
22 20 and 21 (11345)
23 limit 22 to "review" (2010)
24 limit 23 to embase (1656)
25 exp statistics/ (272033)
26 exp regression analysis/ (179182)
27 statistic:.mp. (1196401)
28 (logistic adj2 model:).mp. (31580)
29 (Likelihood adj2 function:).mp. (782)
30 regression:.mp. (461195)
31 exp mathematical phenomena/ (2108262)
32 algorithm:.mp. (176636)
33 mathematic:.mp. (206662)
34 exp multivariate analysis/ (190591)
35 exp biological model/ (805064)
36 statistical model/ (88920)
37 area under the curve/ (55589)
38 or/25-37 (3631278)
39 22 and 38 (5358)
40 limit 39 to embase (4882)
41 24 or 40 (5993)

CINAHL Search Strategy
Tuesday, May 15, 2012 1:44:33 PM

#	Query	Limiters/Expanders	Last Run Via	Results
S29	S18 or S28	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	634
S28	S19 and S27	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	569
S27	S20 or S21 or S22 or S23 or S24 or S25 or S26	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	473798
S26	TX area under curve	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search	116

			Database - CINAHL	
S25	(MH "Models, Theoretical+")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	52897
S24	(MH "Multivariate Analysis") OR (MH "Multivariate Analysis of Variance") OR (MH "Multivariate Analysis of Covariance")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	29451
S23	(MH "Mathematics+")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	291987
S22	TX statistic* or TX logistic N2 model* or TX likelihood N2 function* or TX regression or TX algorithm* or TX mathematic*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	428036
S21	(MH "Regression+")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	109567
S20	(MH "Statistics+")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	282038
S19	S16 and S17	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	1136
S18	S16 and S17	Limiters - Publication Type: Review Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	73
S17	TX mortality or TX survival or TX death	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	158882
S16	S11 and S15	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search	2698

			Database - CINAHL	
S15	S12 or S13 or S14	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	175366
S14	TX ambulatory or TX stable or TX chronic or TX out-patient* or TX outpatient*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	171927
S13	(MH "Outpatients") OR (MH "Outpatient Service")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	29357
S12	(MH "Ambulatory Care") OR (MH "Ambulatory Care Facilities+") OR (MH "Ambulatory Care Nursing")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	13447
S11	S9 and S10	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	8549
S10	S3 or S4 or S5 or S6 or S7 or S8	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	602415
S9	S1 or S2	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	20275
S8	TX "prognostic factor*"	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	2789
S7	(MH "Prognosis+")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	119023
S6	TX evaluation	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	263029

S5	(MH "Risk Factors+")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	62487
S4	(MH "Risk Assessment")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	27594
S3	TX predict* or TX validat* or TX scor* or TX observ*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	276104
S2	TX heart N2 failure or TX cardiac N2 failure	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	20263
S1	(MH "Heart Failure+")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	

Appendix B. Study eligibility form[1]

Reviewer:	XX	ZZ	NN

Article ID:			
Reference #:	Author:	Journal:	Year:

Population[2]:		
• Ambulatory heart failure patients	**YES**	**NO**
• Adults (≥ 19 years old)	**YES**	**NO**

Predictive model[3]:		
• ≥ 2 predictors *or*	**YES**	**NO**
• Validation study of pre-existing model	**YES**	**NO**
• Report of score formula or coefficients	**YES**	**NO**
• Assessment of discrimination and/or calibration	**YES**	**NO**

Outcome reported:		
• Mortality or composite outcome including mortality	**YES**	**NO**
• ≥ 30 deaths	**YES**	**NO**

Study design:		
• Cohort study (prospective or retrospective) *or* • Randomized control trial *or*	**YES**	**NO**

Duplicate population:		
• If duplicated, does this study report new information on model performance?	**YES**	**NO**

Study inclusion:	
• All the answers are YES	**INCLUDE**
• Any answer is NO	**EXCLUDE**

References:

[1] If any response to the above questions is unclear, mark YES.

[2] If a study included hospitalized patients or transplant or VAD patients, consider as NO.

[3] Any type of predictor, including but not limited to clinical characteristics, laboratory values, test results and any other clinical event such as hospital admissions, ICD shocks, etcetera.

SUPPLEMENTAL TABLES

Supplemental Table 1. Assessment of model adequacy and performance

Item	Description
Selection of the predictors	A good model should clearly state how predictors were selected. Potential candidate predictors may be chosen according to correlation with the outcome of interest explored in univariable analysis or based on previous knowledge. Whether one approach is better than the other is a matter of unresolved discussion. The former may include predictors that are not necessarily casual while the latter requires robust knowledge on the field of study.
Coding of the predictors	The proper reporting of the coding of variables is important because the effect of an independent variable on the outcome variable depends on the corresponding units of measurement and the manner in which the variable was coded. Articles were considered to properly report the coding of variables if the method of coding for all of the variables that remained in the final statistical model could easily be determined or were referenced anywhere in the article.
Nonconformity to a Linear Gradient	If the manuscript did not report determining the impact of each explanatory variable separately in zones of ranked data or mentioned that conformity to a linear gradient was addressed, this item was coded as not reported.
Over-fitting	Risk estimates may be unreliable if the multivariable model includes too many independent variables and too few outcome events, they may represent spurious associations or the effects may be estimated with low precision. According to Peduzzi et al [1], we categorized the articles with a ratio of < 10:1 (10 outcome events for each single explanatory variable in the final model) as an over-fitted.

Supplemental Table 1. Continued.

Item	Description
Analysis of statistical model assumption	Violation of model assumptions, such as the proportional hazards assumption in the case of Cox method, may lead to unreliable effect estimates. If a manuscript did not state exploring model assumptions and that they were held in the final proposed model, this item was coded as not reporting model assumptions.
Discrimination	Discrimination expresses to what extent the model is capable of differentiating patients who had the event from those who did not. It is commonly assessed using the c-statistic test, which is equivalent to the area under the receiver operating characteristic (ROC) curve [2]. The ROC curve is a plot of sensitivity versus 1-specificity, which are calculated for each value of the predicted risk as a possible cut-off value. A c-statistic of 0.50 indicates that the model performs no better than chance; a c-statistic of 0.50 to 0.70 indicates poor discrimination; a c-statistic of 0.70 to 0.80 indicates modest discriminative ability; and a c-statistic of greater than 0.80 indicates aceptable discriminative ability [2].
Calibration or goodness of fit	The calibration or goodness of fit of a model measures how well the model describes the response variable. Goodness-of-fit involves investigating how close values predicted by the model are to the observed values. It can be assessed using different methods (i.e., Hosmer-Lemeshow test or deviance, Cox-Snell analysis, correlation between observed vs. predicted events).

References of Supplemental Table 1:

1. Peduzzi P, Concato J, Feinsten AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis II. Accuracy and precision of regression estimates. J Clin Epidemiol 1995;48:1503-10.
2. D'Agostino RB, Byung-Ho Nam. Evaluation of the performance of survival analysis models: Discrimination and calibration measures. In: Handbook of Statistics v23: Advances in survival analysis, by Balakrishnan N, Rao CR. 2004.

Supplemental Table 2. Characteristics of the population of studies included

Study	Model's name	Derivation/ Validation study	Population										Events	
			Source	Inclusion criteria	Time frame	N	Mean Age	% male	Mean LVEF	% ischemic	% β-blocker	% ICD	Definition	n
Aaronson [1] 1997 USA	HFSS	Derivation	Single center	LVEF <40% Age <70 years	1986-1991	268	50	80	20	45	10	n.r.	Death and urgent HTx	109
		Validation	Single centre		1993-1995	199	52	81	22	47	11	n.r.		~60
Zugck [2] 2001 Germany	HFSS	Validation	Single center	NYHA I-III LVEF <40% Age <70 years	1995-1998	208	54	82	22	29	30	n.r.	Death	52
Koelling [3] 2004 USA	HFSS	Validation	Single center	LVEF <40% CP study	1994-1997	320	52	74	23	52	10	11	Death, urgent HTx and VAD	64
					1999-2001	187	54	76	21	56	72	19		30
Parikh [4] 2009 USA	HFSS	Validation	Single center	HF Age >65 years CP study	n.r.	396	70	75	30	50	64	n.r.	Death, urgent HTx and VAD	111
Gorodeski [5] 2010 USA	SHFM HFSS	Validation	Single centre	Referred for HTx assessment	2004-2007	215	55	77	20	55	80	78	Death, urgent HTx and VAD	157

Supplemental Table 2. Continued

Study	Model's name	Derivation/ Validation study	Population										Events	
			Source	Inclusion criteria	Time frame	N	Mean Age	% male	Mean LVEF	% ischemic	% β-blocker	% ICD	Definition	n
Goda [6-8] 2010 USA	HFSS SHFM 3 papers	Validation	Single center	Referred for HTx assessment	1993-2008	715	54	65	22	40	71	49	Death, urgent HTx and VAD	354
Levy [9] 2006 USA	SHFM	Derivation	PRAISE-1 Trial	LVEF <30%	1992-1994	1125	65	76	21	64	0	0	Death, urgent HTx and VAD	403
		Validation[#]	ELITE2 Trial	LVEF <40% Age >60 years	1997-1998	2987	71	69	31	74	24	0		505
			RENAISSANCE trial	LVEF <30% NYHA II-IV	1999-2001	925	62	78	22	61	61	18		179
			Val-HeFT Trial	LVEF <40% NYHA II-IV	1997-1999	5010	63	80	27	58	34	n.r.		979
			IN-CHF Registry	HF patients	1995-n.r.	872	64	76	35	47	35	n.r.		115
			UW Cohort	HF patients	n.r.	148	53	78	27	34	72	22		48

Supplemental Table 2. Continued

Study	Model's name	Derivation/ Validation study	Population										Events	
			Source	Inclusion criteria	Time frame	N	Mean Age	% male	Mean LVEF	% ischemic	% β-blocker	% ICD	Definition	n
May [10] 2007 USA	SHFM	Validation	Single centre	Hospitalized HF patients	1993-2005	4077	67	61	45	60	77	13	Death, urgent HTx and VAD	2142
Allen [11] 2008 USA	SHFM	Validation	Single centre	HF patients	2004-2008	122	61	62	26	38	86	25	Death	35
Kalogeropoulos [12] Giamouzis [13] 2009 USA	SHFM	Validation	Single centre	LVEF <30% NYHA II-IV	2000-2006	445	52	69	18	38	92	68	Death, urgent HTx and VAD	109
Levy [14] 2009 Atlanta, USA	SHFM	Validation	REMATCH trial	HF non-HTx candidates (medical treatment arm)	1998-2001	61	68	82	17	69	20	35	Death	56
Perrota [15] 2012 Italy	SHFM	Validation	Single centre	NYHA I-III LVEF <35% CRT implant	2000-2007	342	71	79	26	52	73	77	Death and urgent HTx	86

Supplemental Table 2. Continued

Study	Model's name	Derivation/ Validation study	Population										Events	
			Source	Inclusion criteria	Time frame	N	Mean Age	% male	Mean LVEF	% ischemic	% β-blocker	% ICD	Definition	n
Haga [16] 2012 UK	SHFM	Validation	Single centre	NYHA III-IV No HF admissions for 6 weeks	n.r.	138	77	66	n.r.	68	59	n.r	Death	43
Frankenstein [17] 2011 Germany	-	Derivation	Single center	LVEF <40%	1995-2005	636	56	81	28	32	78	n.r	Death	151
		Validation			2001-2005	676	74	76	34	63	54	n.r.		160
Kramer [18] 2012 USA	PACE risk score	Derivation	Multi-center	Primary and secondary prevention ICD patients	2001-2008	905	65	78	31	59	n.r.	100	Death	125
		Validation			2001-2008	1812	64	77	31	58	n.r.	100		296
Bilchick [19] 2012 USA	SHOCKED predictors	Derivation	Multi-center (Medicare database)	Primary prevention ICD patients	2005-2006	17991	n.r.	77	n.r.	59	79	100	Death	6741
		Validation			2005-2007	27893	n.r.	75	n.r.	63	n.r.	100		8595

HFSS, Heart Failure Survival Score; LVEF, left ventricular ejection fraction; HTx, heart transplantation; NYHA, New York Heart Association; CP, cardio-pulmonary; VAD, ventricular assist device; SHFM, Seattle Heart Failure Model; MI; myocardial infarction; PRAISE, Prospective Randomized Amlodipine Survival Evaluation; ELITE2, Losartan Heart Failure Survival Study; RENAISSANCE, Randomized Etanercept North American Strategy to Study Antagonism of Cytokines; IN-CHF, Italian Congestive Heart Failure Registry; UW, University of Washington HF clinic; CRT, cardiac resynchronization therapy; HF, heart failure; ICD, internal cardiac defibrillator; n.r., not reported.

Supplemental Table 3. Assessment of study quality

Study	**Derivation Validation**	**Model**	**Patient selection**	**Data collection**	**Missing data**	**Loss of follow up**
Aaronson 1997 [1]	Derivation	**HFSS**	n.r.	Retrospective	n.r.	1-3%
	Validation	**HFSS**	n.r.	Retrospective	n.r.	1-3%
Zugck 2001 [2]	Validation	**HFSS**	n.r.	Retrospective	n.r.	0%
Koelling 2004 [3]	Validation	**HFSS**	n.r.	Retrospective	0%	0%
Parikh 2009 [4]	Validation	**HFSS**	n.r.	Retrospective	36% of patients excluded	0%
Gorodeski 2010 [5]	Validation	**HFSS**	Consecutive	Retrospective	Peak VO_2 = 36%. Imputed by multiple imputation	n.r.
Goda 2010 [6] and 2011 [7,8]	Validation	**HFSS**	Consecutive	Retrospective	18 patients excluded	0%
Levy 2006 [9]	Derivation PRAISE-1	**SHFM**	RCT	Prospective	n.r.	n.r.
	Validation ELITE2	**SHFM**	RCT	Prospective	n.r.	n.r.

Supplemental Table 3. Continued.

Study	**Derivation Validation**	**Model**	**Patient selection**	**Data collection**	**Missing data**	**Loss of follow up**
Levy 2006 [9]	Validation UW	**SHFM**	n.r.	Prospective	n.r.	n.r.
	Validation Val-HeFT	**SHFM**	RCT	Prospective	n.r.	n.r.
	Validation RENAISSANCE	**SHFM**	RCT	Prospective	n.r.	n.r.
	Validation IN-CHF	**SHFM**	Registry	Prospective	n.r.	n.r.
May 2007 [10]	Validation	**SHFM**	Consecutive	Prospective	NYHA=72%, Lymphocytes=35% Uric acid=66%, LVEF=25% Cholesterol=20% Imputed using multiple regression	0%
Allen 2008 [11]	Validation	**SHFM**	Consecutive	Prospective	Imputed with the mean	0%

Supplemental Table 3. Continued

Study	**Derivation Validation**	**Model**	**Patient selection**	**Data collection**	**Missing data**	**Loss of follow up**
Kalogeoropoulos [12] and Giamouzis [13] 2009	Validation	**SHFM**	Consecutive	Retrospective	Exclusion of patients with >2 missing variables. The rest were imputed with the mean (lymphocytes=71%).	0%
Levy 2009 [14]	Validation	**SHFM**	RCT	Prospective	Lymphocytes imputed by multiple regression. Uric acid, cholesterol and diuretic dose were imputed from a comparable group of patients from SHFM cohort.	0%
Gorodeski 2010 [5]	Validation	**SHFM**	Consecutive	Retrospective	Uric acid = 64%, Cholesterol = 11% Lymphocytes = 10% Imputed by multiple imputation	n.r.
Goda 2011 [8]	Validation	**SHFM**	Consecutive	Retrospective	In 38% patients, imputed with the mean	0%
Perrota 2012 [15]	Validation	**SHFM**	n.r.	Retrospective	Imputed with the mean	n.r.

Supplemental Table 3. Continued.

Study	**Derivation Validation**	**Model**	**Patient selection**	**Data collection**	**Missing data**	**Loss of follow up**
Haga 2012 [16]	Validation	**SHFM**	n.r.	Retrospective	n.r.	n.r.
Frankenstein 2011[17]	Derivation	-	Consecutive	Retrospective	n.r.	n.r.
	Validation		Consecutive	Retrospective	n.r.	n.r.
Kramer 2012 [18]	Derivation	**PACE risk score**	Consecutive	Retrospective	n.r.	n.r.
	Validation		Consecutive	Retrospective	n.r.	n.r.
Bilchick 2012 [19]	Derivation	**SHOCKED predictors**	Consecutive	Prospective	n.r.	n.r.
	Validation		Consecutive	Prospective	n.r.	n.r.

HFSS, Heart Failure Survival Score; peak VO_2, peak oxygen consumption; RCT, randomized controlled trial; SHFM, Seattle Heart Failure Model; PRAISE, Prospective Randomized Amlodipine Survival Evaluation; ELITE2, Losartan Heart Failure Survival Study; RENAISSANCE, Randomized Etanercept North American Strategy to Study Antagonism of Cytokines; IN-CHF, Italian Congestive Heart Failure Registry; UW, University of Washington HF clinic; LVEF, left ventricular ejection fraction; n.r., not reported.

References of Supplemental Tables 2 and 3:

1. Aaronson KD, Schwartz JS, Chen T-M, Wong K-L, Goin JE, Mancini DM. Development and prospective validation of a clinical index to predict survival in ambulatory patients referred for cardiac transplant evaluation. Circulation 1997;95:2660–7.
2. Zugck C, Kruger C, Kell R, Korber S, Schellberg D, Kubler W, Haass M.. Risk stratification in middle-aged patients with congestive heart failure: prospective comparison of the Heart Failure Survival Score (HFSS) and a simplified two-variable model. Eur J Heart Fail 2001;3:577-85.
3. Koelling TM, Joseph S, Aaronson KD. Heart Failure Survival Score continues to predict clinical outcomes in patients with heart failure receiving β-blockers. J Heart Lung Transplant 2004;23:1414-22.
4. Parikh MN, Lund LH, Goda A, Mancini D. Usefulness of peak exercise oxygen consumption and the heart failure survival score to predict survival in patients >65 years of age with heart failure. Am J Cardiol 2009;103:998-1002.
5. Gorodeski EZ, Chu EC, Chow CH, Levy WC, Hsich E, Starling RC. Application of the Seattle Heart Failure Model in ambulatory patients presented to an advanced heart failure therapeutics committee. Circ Heart Fail 2010;3:706-14.
6. Goda A, Lund LH, Mancini D. Comparison across races of peak oxygen consumption and heart failure survival score for selection for cardiac transplantation. Am J Cardiol 2010; 105:1439-44.
7. Goda A, Lund LH, Mancini D. The Heart Failure Survival Score outperforms the peak oxygen consumption for heart transplantation selection in the era of device therapy. J Heart Lung Transplant 2011;30:315-25.
8. Goda A, Williams P, Mancini D, Lund LH. Selecting patients for heart transplantation: comparison of the Heart Failure Survival Score (HFSS) and the Seattle heart failure model (SHFM). J Heart Lung Transplant 2011;30:1236-43.
9. Levy WC, Mozaffarian D, Linker DT, Sutradhar SC, Anker SD, Cropp AB, Anand I, Maggioni A, Burton P, Sullivan MD, Pitt B, Poole-Wilson PA, Mann DL, Packer M. The Seattle Heart Failure Model: prediction of survival in heart failure. Circulation 2006;113:1424–33.
10. May HT, Horne BD, Levy WC, Kfoury AG, Rasmusson KD, Linker DT, Mozaffarian D, Anderson JL, Renlund DG. Validation of the Seattle Heart Failure Model in a community-based heart failure population and enhancement by adding B-type natriuretic peptide. Am J Cardiol 2007;100:697-700.
11. Allen LA, Yager JE, Funk MJ, Levy WC, Tulsky JA, Bowers MT, Dodson GC, O'Connor CM, Felker GM. Discordance between patient-predicted and model-

predicted life expectancy among ambulatory patients with heart failure. JAMA 2008;299:2533-42.

12. Kalogeropoulos AP, Georgiopoulou VV, Giamouzis G, Smith AL, Agha SA, Waheed S, Laskar S, Puskas J, Dunbar SA, Vega, D, Levy WC, Butler J. Utility of the Seattle Heart Failure Model in patients with advanced heart failure. J Am Coll Cardiol 2009;53:334-42.
13. Giamouzis G, Kalogeropoulos AP, Georgiopoulou VV, Agha SA, Rashad MA, Laskar SR, Smith AL, Butler J. Incremental value of renal function in risk prediction with the Seattle Heart Failure Model. Am Heart J 2009;157:299-305.
14. Levy WC, Mozaffarian D, Linker DT, Farrar DJ, Miller LW, REMATCH Investigators. Can the Seattle heart failure model be used to risk-stratify heart failure patients for potential left ventricular assist device therapy? J Heart Lung Transplant 2009;28:231-6.
15. Perrotta L, Ricciardi G, Pieragnoli P, Chiostri M, Pontecorboli G, De Santo T, Bellocci F, Vitulano N, Endin M, Mascioli G, Ricceri I, Porciani MC, Michelucci A, Padeletti L. Application of the Seattle Heart Failure Model in patients on cardiac resynchronization therapy. PACE 2012;35:88-94.
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18. Kramer DB, Friedman PA, Kallinen LM, Morrison TB, Crusan DJ, Hodge DO, Reynolds MR, Hauser RG.. Development and validation of a risk score to predict early mortality in recipients of an implantable Cardioverter-defibrillator. Heart Rhythm 2012;9:42-6.
19. Bilchick KC, Stukenborg GJ, Kamath S, Cheng A. Prediction of mortality in clinical practice for Medicare patients undergoing defibrillator implantation for primary prevention of sudden cardiac death. J Am Coll Cardiol 2012;60:1647-55.

Supplemental Table 4. Characteristics of the population of studies included.

Study	Model name	Derivation /Validation study	Population										Events	
			Source	Inclusion criteria	Time frame	N	Mean Age	% male	Mean LVEF	% ischemic	% β-blocker	% ICD	Definition	n
Kearney 2003 [1] UK	-	Derivation	Heart study	Clinically diagnosed HF	1993-1995	553	63	76	42	79	8	n.r.	Death	201
Rickli 2003 [2] Switzerland	-	Derivation	Single center	LVEF<40% CP study	n.r.	202	52	86	28	53	45	n.r	Death and urgent HTx	59
Adlam 2005 [3] UK	-	Derivation	Single centre	Clinically diagnosed HF	1995-1998	532	75	41	45	41	14	n.r.	Death	190
Pocock 2006 [4] UK	CHARM	Derivation	CHARM trial	Clinically diagnosed F	1999-2003	7599	65	68	39	57	n.r.	n.r.	Death	1831
Myers 2008 [5] Italy	CPX score	Derivation	Multi-center	Clinically diagnosed HF	1993-2007	710	56	80	34	39	63	n.r.	Death, urgent HTx and VAD *	110

Supplemental Table 4. Continued.

Study	Model name	Derivation /Validation study	Population										Events	
			Source	Inclusion criteria	Time frame	N	Mean Age	% male	Mean LVEF	% ischemic	% β-blocker	% ICD	Definition	n
Huynh 2008 [6] USA	-	Derivation	Single center	HF patients Age >70 years	1990-1994	282	80	34	42	54	n.r.	n.r.	Death	43
Wedel 2009[7] Europe	CORONA score	Derivation	CORONA trial	LVEF <40% NYHA II-IV	2003-2005	3342	72	73	32	100	78	2.3	Death *	934
Leyva 2009 [8] UK	DSC index	Derivation	Single center	LVEF<35% NYHA III-IV CRT implant	2001-2008	148	68	77	23	62	55	0	CV Death	37
Vazquez 2009 [9] Spain	MUSIC score	Derivation	Multi-centre	Clinically diagnosed HF NYHA II-IV	2003-2004	992	65	72	37	46	68	n.r.	Death *	267
Komajda 2011 [10] France	-	Derivation	I-PRESERVE trail	LVEF >45% NYHA II-IV Age >50 years	2003-2007	4128	72	40	59	25	n.r.	n.r.	Death *	881

Supplemental Table 4. Continued

Study	Model's name	Derivation /Validation study	Population										Events	
			Source	Inclusion criteria	Time frame	N	Mean Age	% male	Mean LVEF	% ischemic	% β-blocker	% ICD	Definition	N
Subramanian 2011 [11] USA	VEST score	Derivation	VEST trail	LVEF <30% NYHA III-IV	1995-1996	963	62	78	21	57	n.r.	n.r.	Death *	172
O'Connor 2012 [12] USA	HF-ACTION score	Derivation	HF-ACTION trail	LVEF <35% NYHA II-IV	2003-2007	2331	59	72	25	54	95	40	Death *	387
Herrmann 2012 [13] UK		Derivation	Single centre	LVEF <40% HF symptoms	n.r.	114	63	n.r.	29	n.r.	4	n.r.	Death	31
Scrutinio 2012 [14] Italy		Derivation	Single centre	LVEF <40% HF symptoms	2001-2007	802	64	79	28	50	73	n.r.	Death	301
Pocock 2012[15] Europe		Derivation	Multi-centre	Clinically diagnosedHF	n.r.	39372	67	67	35	53	34	n.r.	Death	15851

HF, heart failure; NYHA, New York Heart Association; CP, cardio-pulmonary; LVEF, left ventricular ejection fraction; HTx, heart transplantation; VAD, ventricular assist device; CV, cardiovascular; n.r., not reported.

Supplemental Table 5. Assessment of study quality

Study	**Derivation Validation**	**Model**	**Patient selection**	**Data collection**	**Missing data**	**Loss of follow up**
Kearney 2003 [1]	Derivation		n.r.	Prospective	Multiple regression	n.r.
Rickli 2003 [2]	Derivation		Consecutive		n.r.	n.r.
Adlam 2005 [3]	Derivation		Consecutive	Prospective	Excluded	0%
Pocock 2006 [4]	Derivation	**CHARM**	RCT cohort	Prospective	n.r.	n.r.
Myers 2008 [5]	Derivation	**CPX score**	n.r.	Prospective	n.r.	n.r.
Huynh 2008 [6]	Derivation		RCT cohort	Prospective	n.r.	n.r.
Wedel 2009 [7]	Derivation	**CORONA**	RCT cohort	Prospective	Excluded	n.r.
Leyva 2009 [8]	Derivation	**DSC index**	Consecutive	Prospective	0%	0%
Vazquez 2009 [9]	Derivation	**MUSIC score**	Consecutive	Prospective	Imputed with the mean	1.1%
Komajda 2011 [10]	Derivation		RCT cohort	Prospective	Excluded	n.r.
Subramanian 2011 [11]	Derivation	**VEST**	RCT cohort	Prospective	19% of patients excluded	n.r.

Supplemental Table 5. Continued

Study	Derivation Validation	Model	Patient selection	Data collection	Missing data	Loss of follow up
O'Connor 2012 [12]	Derivation	**HF-ACTION**	RCT cohort	Prospective	Hemoglobin 24%, Urea 13% Sodium 11%, Creatinine 10% MR 8% Multiple imputation	n.r.
Herrmann 2012 [13]	Derivation		n.r.	Prospective	n.r.	n.r.
Scrutinio 2012 [14]	Derivation		Consecutive	Prospective	0%	0%
Pocock 2012 [15]	Derivation		Meta-analysis on RCT and observational studies	Prospective and retrospective	Multiple imputation	0%

LVEF, left ventricular ejection fraction; ICD, internal cardiac defibrillator; HFSS, Heart Failure Survival Score; HTx, heart transplantation; VAD, ventricular assist device; NYHA, New York Heart Association; MFH; metabolic, functional, hemodynamic; CPX, cardiopulmonary exercise test; MRT, mean response time; SHFM, Seattle Heart Failure Model; MI; myocardial infarction; DSC, Dyssynchrony, posterolateral Scar location and Creatinine; CRT, cardiac resynchronization therapy; CV, cardiovascular; n.r., not reported.

Supplemental Table 6. Model derivation and performance

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
Zugck 2001 [15]	Derivation	• LVEF • Peak VO_2 or 6'WT	n.r.	n.r.	No	n.r.	n.r.	Overall = 0.84 (0.80-0.88) or 0.83 (0.79-0.87)
Kearney 2003 [1]	Derivation	• Sodium • Creatinine • CT ratio • QRS dispersion • QT • Non-sustained VT • LVH by ECG • SDNN	Based on univariable analysis	n.r.	Yes (201 events and 30 variables tested)	Held	n.r.	* Binary predictors= 0.74 (0.70-0.78) Continuous predictors= 0.78 (0.74-0.82)
	Validation by bootstrap	**Kearney 2003**	n/a	n/a	n/a	n/a	n.r	n.r.
Rickli 2003 [2]	Derivation	• Predicted peak VO_2 • MRT >50 seconds • Systolic BP	Based on univariable analysis	n.r.	No	n.r.	n.r.	At 1 year=0.86 (0.82-0.90)

Supplemental Table 6. Continued.

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
Adlam 2005 [3]	Derivation	• BNP • Age • Sex • Diabetes • CVA • Abnormal ECG	Based on univariable analysis using bootstrap estimated	n.r.	No	Held	n.r.	Overall = 0.76
	Validation by bootstrap	**Adlam** • **2005**	n/a	n/a	n/a	n/a	n.r.	Overall = 0.75

Supplemental Table 6. Continued.

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
Pocock 2006 [4]	Derivation	**CHARM:** • Age • Sex • Diabetes • LVEF • NYHA • Cardiomegaly • Time HF diagnose • Prior admission • BMI • Diastolic BP • Smoking • BBB • Previous MI • Crackles • Edema • Pulmonary edema • Heart Rate • Mitral regurgitation • Atrial fibrillation • Rest dyspnea • Candesartan	Probably on clinical importance Forward selection	n.r.	No	n.r.	Graphically observed vs. predicted survival by deciles. Under-estimated survival at 3 years	At 2 years = 0.75 In preserved EF = 0.74 In low-EF=0.76

Supplemental Table 6. Continued.

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
	Validation by bootstrap	**CHARM**	n/a	n/a	n/a	n/a	n.r.	At 2 years = 0.75
Myers 2008 [5]	Derivation	**CPX score:** • OUES>1.4 • VE/VCO2 >34 • peak VO2<14 • HR recovery <6 beats at 1minute • PetCO2 <33mmHg	Not clearly stated	n.r.	No	Held	n.r.	n.r.
	Validation by bootstrap	**CPX score**	n/a	n/a	n/a	n/a	n.r.	‡ Overall = 0.77
Huynh 2008 [6]	Derivation	• Urea • Systolic BP • PVD • Sodium	Based on univariable analysis.	n.r.	Yes (43 events and 15 variables)	n.r.	n.r.	At 6 months=0.80

Supplemental Table 6. Continued.

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
	Validation by bootstrap	**Huynh 2008**	n/a	n/a	n/a	n/a	n.r.	n.r.
Wedel 2009 [7]	Derivation	**CORONA:** • BNP • Age • Diabetes • LVEF • BMI • Sex • CABG • Atrial fibrillation • NHYA • Apo-A1 • Creatinine • PVD • Heart rate • MI	Not clearly stated	n.r.	No	n.r.	n.r.	Overall mortality=0.72 HF mortality=0.80

Supplemental Table 6. Continued.

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
Leyva 2009 [8]	Derivation	**DSC index:** • Dyssynchrony • Scar location • Creatinine	Based on previous reports	Checked by martingale residuals	No	Held	Correlation (r=0.93)	At 1 year = 0.88 At 1 year = 0.87
	Validation by bootstrap	**DSC index**	n/a	n/a	n/a	n/a	****	Overall=0.85
Vazquez 2009 [9]	Derivation	**MUSIC score:** • Prior MI, stroke or limb ischemia • Left atrium size>26mm/m2 • LVEF<35% • LBBB or IVCD (QRS>110) • non-sustained VT or frequent extra-beats • GFR <60ml/min • BNP>1000pg/dl • Troponin posit • Sodium <138meq/L	Based on previous knowledge and <5% missing data	n.r.	No	n.r	Correlation (r=0.99)	Overall mortality=0.76 Cardiac mortality=0.78 HF mortality=0.80 Sudden death=0.77

Supplemental Table 6. Continued.

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
	Validation by bootstrap	**MUSIC score**	n/a	n/a	n/a	n/a	n.r.	Overall mortality=0.77 Cardiac mortality=0.78 HF mortality=0.80 Sudden death=0.78
Komajda 2011 [10]	Derivation	• BNP • Age • Diabetes • LVEF • Heart rate • Previous hospital admission • Quality of life • COPD or asthma • Ischemic CMP • MI	Based on univariable analysis	n.r.	No	n.r.	Graphically observed vs. predicted = Adequate	Overall=0.74
	Validation by bootstrap	**Kornajda 2011**	n/a	n/a	n/a	n/a	n.r.	Overall=0.74

Supplemental Table 6. Continued.

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
Subramanian 2011 [11]	Derivation	**VEST:** Model:1 • BUN • LVEF • Lymphocytes • CT radio Model 2: 1+ • TNFR • Interleukin 6 Model 3: 2+ • Serial measurement of cytokines	Based on univariable analysis	n.r.	Yes (172 events and 19 variables tested)	n.r.	n.r.	Overall= Model 1: 0.73 Model 2: 0.74 Model 3: 0.81

Supplemental Table 6. Continued.

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
O'Connor 2012 [12]	Derivation	**HF-ACTION:** • Exercise duration • Urea • Sex • BMI	Based on univariable analysis	Checked by restrictive cubic spline	No	n.r.	Correlation (r=0.99 at 1,2 and 3 years and 0.98 at 5 years)	Overall=0.73
Herrmann 2012 [13]	Derivation	• Peak VO_2 <14ml/kg/min • Uric acid >565µmol/L • LVEF<22% • Cholesterol <5.27mmol/L • sTNF-R1 >1016pg/L	Based on previous knowledge	n.r.	Yes (31 deaths and 5 variables tested)	n.r.	n.r.	† Overall=0.91

Supplemental Table 6. Continued.

Study	Derivation Validation	Model/ Variables	Selection	Linear Gradient	Over-fitting	Model assumptions	Calibration	Discrimination (c-statistic)
Scrutinio 2012 [14]	Derivation	• Age • Ischemic CMP • Anemia • LVEF • Renal function	Based on univariable analysis	n.r.	No	n.r.	H-L test (p>0.45)	Overall=0.74
Pocock 2012 [15]	Derivation	• Age • Gender • BMI • Current smoker • Systolic BP • Diabetes • NYHA class • LVEF • COPD • HF duration • Creatinine • β-blockers • ACE-I/ARB	Based on statistical significance	n.r.	No	n.r.	Graphically observed vs. predicted = Adequate	n.r.

* This model was validated by bootstrapping but discrimination capacity on bootstrapping is not reported.

‡ Authors conducted a subgroup analysis based on underlying etiology and LVEF and reported that c-index was equal in ischemic, non-ischemic CMP and patients with LVEF <30%, but lower (c-statistic = 0.73) in patients with LVEF ≥30%.

† Authors reported that a model excluding cholesterol has similar c-statistic and that a model including uric acid, sTNF-R1, LVEF and NYHA class (<3) instead of peak VO_2 had an overall c-statistic of 0.84.

LVEF, left ventricular ejection fraction; VO2, oxygen consumption; CT, cardio-thoracic; VT, ventricular taqui-arrhythmia; LVH, left ventricular hypertrophy; ECG, electro-cardiogram; SDNN, standard deviation of all R-to-R intervals on 24-h; MRT, mean response time; BP, blood pressure; CVA, cerebro-vascular accident; NYHA, New York Heart Association; BMI, body mass index; BBB, bundle branch block; MI, myocardial infarction; PVD, peripheral vascular disease; ICD, internal cardiac defibrillator; MFH; metabolic, functional, hemodynamic; CPX, cardiopulmonary exercise test; MRT, mean response time; MI; myocardial infarction; DSC, Dyssynchrony, posterolateral Scar location and Creatinine; CRT, cardiac resynchronization therapy; CV, cardiovascular; BNP, brain natriuretic peptide; COPD, chronic obstructive pulmonary disease; CMP, cardiomyopathy; sTNF-R1, soluble tumor necrosis factor alpha receptor 1; H-L, Hosmer and Lemeshow; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; n.r., not reported; n/a, not applicable.

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CHAPTER III

The work described in this chapter has accepted for publication in the Canadian Journal of Cardiology. (Alba et al. Can J Cardiol 2013; in press)

Title: Predictors of mortality in patients with an implantable cardiac defibrillator: A systematic review and meta-analysis

Short title: Mortality predictors in ICD patients

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BRIEF SUMMARY

Previously recognized prognostic factors may not have the same predictive value in ICD heart failure patients. Through a meta-analysis, we identified age, renal function, COPD, diabetes, peripheral vascular disease, LVEF and ICD shocks as strong independent predictors of mortality with high confidence in estimates. NYHA class was strongly associated with mortality but the confidence in estimates was low. Ischemic cardiomyopathy and sex were not mortality predictors. Limited evidence on peakVO_2 and laboratory markers precluded analysis.

ABSTRACT

Background: Many current predictors of mortality in heart failure (HF) were evaluated before the use of implantable cardioverter defibrillators (ICD). We conducted a meta-analysis to identify factors associated with mortality in HF patients with ICD.

Methods: We searched for studies in MEDLINE, EMBASE and CINAHL in May 2012. Two reviewers selected citations including ambulatory ICD patients, addressing the association between any predictor and mortality using multivariable regression. We meta-analyzed mortality using random-effects models.

Results: Of 10,420 studies reviewed, 72 studies evaluating 63 predictors on 257,692 ICD patients proved eligible. High confidence in estimates was found for age (HR 1.45 for 10-year increase, 95%CI 1.35-1.56), baseline glomerular filtration rate (HR 1.25 for15-ml/min decrease, 95%CI 1.15-1.35), chronic obstructive pulmonary disease (HR 1.54, 95%CI 1.38-1.71), diabetes (HR 1.56, 95%CI 1.37-1.79), peripheral vascular disease (HR 1.43, 95%CI 1.2-1.72), left ventricular ejection fraction (HR 0.77 for 10% increase, 95%CI 0.73-0.83) and occurrence of appropriate or inappropriate ICD shocks (HR 2.34, 95%CI 1.59-3.44). NYHA class, atrial fibrillation and congestive HF were strongly associated with mortality but the confidence in estimates was low to very low. Ischemic cardiomyopathy and male sex were not independent predictors of mortality.

Conclusions: This meta-analysis identified strong reliable mortality predictors in ICD-HF patients. Older age, renal dysfunction, history of COPD, diabetes and PVD, decreased LVEF and the occurrence of shocks during follow-up were strong predictors of mortality;

ischemic cardiomyopathy and male sex were not. Further research is needed to study other potential predictors, particularly biomarkers. .

INTRODUCTION

Heart failure (HF) is a growing health problem, with high morbidity and mortality, increasing prevalence, and rising costs [1,2]. Optimal management of HF relies on accurate estimation of prognosis. However, predicting patients' clinical course is difficult. Older age, multiple co-morbidities, and different patterns of disease progression create important challenges in predicting prognosis. Moreover, changes in therapies and patient management over time may modify the impact of prognostic factors on the clinical course of HF.

The increased use of implantable cardioverter defibrillators (ICD) represents one important change in the management of HF patients. Currently, ICD's are part of the standard management of patients with HF. The American (ACC/AHA and HRS) and European (ESC and EHRA) Societies of Cardiology and Heart Rhythm have recently updated their guidelines regarding ICD indications [3-5]. Based on the results of recent trials, current indications may be summarized in two categories: primary prevention, in HF patients on optimal medical treatment, with symptomatic ischemic or non-ischemic cardiomyopathy (CMP), with reduced left ventricular ejection fraction (LVEF); and secondary prevention, in patients with confirmed or highly suspicious life-threatening tachyarrhythmia. The results of trials and consequent guideline modifications have changed practice in many countries reflecting in an increase of more than 100% in the use of ICD between 2003 and 2005 in North America and Europe [6].

The intention of ICD therapy is to prevent sudden cardiac death. The main indication for ICD is primary prevention (82% of ICDs implanted in the USA, and approximately 55% in Europe) [7].

Previously recognized prognostic factors may not have the same predictive value in HF patients who have recently received an ICD. In addition, most of currently used prognostic models were developed in non-ICD cohorts [8]. The vast amount of information on prognostic factors in heart failure makes it difficult to incorporate all available evidence into healthcare decisions and the conduction of research. We therefore conducted a systematic review and meta-analysis to identify factors associated with mortality in ICD patients and to assess the magnitude of these associations. A systematic review summarises all available evidence minimizing bias, providing reliable findings and identifying gaps in our knowledge.

METHODS

Data sources and searches: In May 2012, with the assistance of an experienced research librarian, we conducted a systematic search of electronic databases, including Medline, Cochrane, Embase and CINAHL. We used several related terms: ("implantable cardiac defibrillator) AND ("heart" OR "cardiac") AND ("mortality" OR "survival") AND ("multivariate analysis" OR "regression analysis" OR "risk factor" OR "prediction" OR "prognostic factor"). The full search strategy is shown in Appendix A in Supplemental Methods. We identified additional studies by searching bibliographic references of included publications.

Study selection: Eligible articles enrolled adult (>19 years) ambulatory ICD patients, evaluated any factor associated with mortality, used multivariable analysis (with at least three independent variables) and reported more than 30 deaths. We included retrospective and prospective cohort studies and *post hoc* analysis of randomized control trials (RCTs). There were no restrictions based on primary or secondary ICD indications, language or date of publication. We excluded studies that enrolled patients during a hospital admission, or duplicate studies providing no new relevant data.

Two reviewers independently screened titles and abstracts and then evaluated full-text versions of all articles deemed potentially relevant by either reviewer. During full text screening, in cases of disagreement, consensus was reached through discussion. If consensus could not be reached, a third reviewer resolved the issue. Agreement between reviewers was assessed using weighted kappa (0.88). Appendix B in Supplemental Methods shows the eligibility form.

Data abstraction and quality assessment: Data abstraction was performed in duplicate using a structured form. We abstracted data related to eligibility criteria, data source, time frame of recruitment and characteristics of the population, including age, sex, ischemic CMP, LVEF, ICD indication, use of β-blockers and cardiac resynchronization therapy (CRT), definition and number of events. We also identified variables included in the prediction models, definitions of predictors and their effect sizes.

Quality assessment was performed at the study level (risk of bias in individual studies) and at the predictor level (confidence in the entire body of evidence for individual predictors). Items for risk of bias assessment were based on recommendations

by Moons et al [9] and customized for this study. We abstracted variables to appraise study quality, including consecutive patient selection, study design (prospective versus retrospective design), loss to follow up, predictors selection process (based on statistical or clinical significance and full or reduced models), missing data related to predictors, conformity with linearity for continuous predictors, model assumptions, model overfitting and validation of results [10]. Appendix C in Supplemental Methods shows a detailed description of these items.

We assessed the confidence in the entire body of evidence based on the sensitivity analyses. We used a modified version of the GRADE approach to assessing confidence in estimates across studies at each predictor level (Appendix D in Supplemental Methods). We used risk of bias assessment at the study level to inform the risk of bias at the body of the evidence level along with inconsistency, imprecision, indirectness and reporting bias as factors that decrease the confidence in estimates; and large effect and gradient response as factors that increase confidence. Risk of selective reporting bias, due to the unreported effect of factors non-significantly associated with mortality, was minimized through a comprehensive search for potential studies and by contacting authors of included studies. We contacted 36 authors to obtain unreported HR and 95%CI of variables included in final models; 6 of 36 responded. We formally assessed selective reporting bias with funnel plots at different stages in the analysis. We summarized confidence in estimates as high, moderate, low, or very low.

Data synthesis and statistical analysis: All studies evaluated the association between predictors and mortality using Cox proportional hazards models and reported

hazards ratios (HR) and their respective 95% confidence intervals (95%CI). We meta-analyzed these results when the effect estimates associated with a specific predictor were reported in more than one study using a similar definition. We estimated pooled HR estimates for each predictor using the inverse variance method through random-effects analysis. In order to summarize the results of these analyses, we grouped these predictors into demographic factors, co-morbidities, factors associated with HF characteristics and factors associated with ICD therapy. We excluded from this report predictors associated with therapies (i.e. use of β-blockers, diuretics, digoxin, CRT, etcetera).

We conducted separate analyses when a continuous predictor, such as age, renal function or LVEF, was analyzed as both continuous and categorical variable. When the predictor was categorized as a binary variable, we conducted a subgroup analysis to test for the presence of an interaction effect. To incorporate studies with discrete variables with more than 2 categories into models where we wished to treat those variables as continuous, we calculated the HR and corresponding 95%CI associated with a unit change in the predictor by averaging the coefficients and variances for each category and then pooling those averages across studies.

We evaluated heterogeneity using the I^2 statistic, which estimates the proportion of total variation associated with between-study variation; in other words, heterogeneity across studies. The extent of heterogeneity was judged based on recommendations from the Cochrane Collaboration [11]: 0-40% represents unimportant heterogeneity, 30-60% moderate, 50-90% substantial and 75-100% considerable heterogeneity; the overlapping boundaries acknowledge the judgment that is required in the interpretation; therefore,

when results fall in or near the overlapping parts of these categories, we considered the likelihood of under- or over-powered estimates and the significance of the extent of variability in regard to confidence in estimates.

We conducted a series of sensitivity analyses specified *a priori* to identify potential sources of heterogeneity, while excluding post-hoc analysis on RCT population to ensure a better representation of general HF population, studies with over-fitted models and studies including fewer than 4 strong predictors in their final models. We identified strong predictors using the baseline pooled estimates. In the case of categorical variables, the presence of a HR of at least 1.5 (or 0.67 in the case of an inverse association) and a ratio of the natural logarithmic function of the HR and its standard error >3.5 was considered as indicating a strong association between the predictor and mortality. In the case of a continuous variable, a ratio of the natural logarithmic function of the HR and its standard error >3.5 was considered as indicating a strong association.

A two-sided p-value of 0.05 or lower was considered statistically significant. Review Manager 5 was used to perform data analysis and to generate graphs.

RESULTS

Study selection and characteristics

Supplemental Figure S1 shows the flow diagram of study selection. We included 72 studies involving 257,692 ICD patients. Supplemental Table S1 summarizes the characteristics of these studies. Eight studies included only secondary prevention ICD patients, 15 studies included only primary prevention ICD patients, 36 studies included

both primary and secondary prevention and 13 studies did not report the ICD indication. All but 3 studies reported on all cause mortality; 2 reported on deaths due to progressive HF and 1 on cardiac deaths.

Study era ranged from 1980 to 2010. The vast majority of patients were included in studies with a recruitment date after 2000 (231,399 patients); only 2,910 patients were recruited in studies before 2000. The characteristics of the population of the studies included showed a mean age from 65 to 72 years, between 72 and 87% were male, mean LVEF varied from 21 to 38%, between 55 and 72% had ischemic CMP, 58 to 78% were on β-blockers and between 50 to 91% of the patients had a primary-prevention ICD.

Risk of bias at individual studies

Supplemental Table S2 summarizes the quality assessment of individual studies. Overall, the risk of bias of individual studies was low to moderate. All studies included consecutive patients. A majority (52%, 37 of 72 studies) had a prospective or retrospective cohort design on prospectively collected data and included most of the patients in this analysis. There were 11 prospective cohort studies (190,393 patients) and 27 retrospective studies on prospectively collected data (39,095 patients). Of the latter, 17 studies analyzed patients enrolled in previous RCTs (8,006 patients) and 35 retrospective cohort studies (28,204 patients).

The selection of candidate predictors included in the models was based on clinical importance in 33 studies, statistical significance of univariable analysis in 29 studies and not reported in 10 studies. Overfitting was infrequent among the included studies; only 15

studies of 72 studies had overfitting. Only one study validated their results using bootstrapping.

Other items used for study risk of bias assessment were often poorly reported; 73% of studies did not report on the frequency of loss to follow-up, 76% did not report the presence of missing information, 85% did not report the assessment of model assumptions and 93% did not report the evaluation of linearity of the association between continuous predictors and the outcome. This under-reporting suggests the possibility of bias.

Predictors

We identified a total of 63 predictors (cited in Supplemental Table S1) evaluated in the included studies and meta-analyzed 18 predictors that were evaluated in more than one study. Figure 1 and Supplemental Table S3 summarize the main findings of these analyses.

Demographic predictors

Among demographic factors, we identified age, sex and race. The impact of age on mortality was reported in 31 studies. When age was evaluated as a continuous variable, a 10-year increase in age was associated with 45% increase in hazard (HR 1.45, 95%CI 1.35-1.56, I^2=49%). When evaluated as a categorical variable, age persisted as a factor significantly associated with mortality and the effect estimate increased with higher cut-offs (at 65 years, HR 1.5, 95%CI 1.28-1.76; at 70 years, HR 1.97, 95%CI 1.6-2.43; at 75 years, HR 2.7, 95%CI 1.48-4.92). The subgroup analysis comparing groups with

different cut-offs suggested a gradient of higher mortality risk at older age (p = 0.10) (Figure 2).

Sex was addressed in 13 studies. The pooled estimate showed that male sex was not associated with higher mortality (HR 1.17, 95%CI 0.86-1.59, I^2=70%). The analysis of race included 3 studies showing that non-white race was associated with higher mortality (HR 1.23, 95%CI 1.08-1.41, I^2=34%). Supplemental figures S2 and S3 show these results.

Age as a continuous variable and non-white race warranted high or high to moderate confidence in estimates; age analyzed as a categorical variable and male sex warranted moderate confidence. The main limitations were the risk of bias at the study level due to the use of statistical significance from univariable analysis for variable selection, and imprecision in the pooled estimates (Table 1).

Co-morbidities

Co-morbidities included were chronic renal dysfunction (CRD), diabetes, hypertension, chronic obstructive pulmonary disease (COPD) and peripheral vascular disease (PVD).

The impact of CRD as a mortality predictor was reported as a binary variable with different cut-offs of a pre-specified glomerular filtration rate (GFR) or creatinine and/or need for dialysis in 16 studies; 10 studies used GFR (7 studies) or creatinine (3 studies) at baseline as a continuous variable. In studies using CRD as a categorical variable, we conducted two subgroup analyses by dividing the studies on the presence of moderate CRD (using as cut-off GFR of 60ml/min or creatinine of ~1.5 mg/dL) or severe CRD

(using as cut-off GFR 30ml/min or creatinine of 2 mg/dL or need for dialysis). Based on the sensitivity analysis, both moderate and severe CRD were significantly associated with higher mortality risk (HR 1.7, 95%CI 1.32-2.19, I^2=87% and HR 2.72, 95%CI 1.45-4.22, I^2=75%, respectively). These estimates were not significantly different (p=0.72). Our confidence in estimates was very low due to the presence of risk of bias at the study level for the use of univariable analysis for variable selection, unexplained inconsistency, indirectness related to the use of different definitions, imprecision and risk of reporting bias.

When CRD was analyzed as a continuous predictor using baseline GFR, a 15-ml/min decrease was associated with 25% increase in hazard (HR 1.25, 95%CI 1.15-1.35, I^2=0%) (Figure 3). Creatinine at baseline was also a significant mortality predictor, with a 1-mg/dL increase being associated with 28% increase in hazard (HR 1.28, 95%CI 1.11-1.49, I^2=0%) (Supplemental Figure S4). GFR as a continuous variable was associated with high quality of the evidence while creatinine presented moderate quality of the evidence due to risk of bias at the study level.

Other co-morbidities associated with mortality were diabetes (HR 1.56, 95%CI 1.37-1.79, I^2=61%), COPD (HR 1.54, 95%CI 1.38-1.71, I^2=36%) and PVD (HR 1.43, 95%CI 1.2-1.72, I^2=61%). Hypertension was not a significant predictor of mortality (HR 1.26, 95%CI 0.46-3.43, I^2=94%). These results are shown in Supplemental Figures S5-S8. Evidence for COPD and PVD warranted high confidence; for diabetes, moderate, due to the risk of bias and reporting bias. Evidence regarding hypertension warranted only

very low confidence due to the additional presence of unexplained inconsistency and imprecision (Table 1).

Factors associated with HF characteristics

Within this category, we grouped predictors associated with HF severity, including New York Heart Association (NYHA) class, LVEF and history of congestive HF or symptomatic HF; the presence of ischemic CMP; atrial fibrillation and QRS duration. Figure 1 and Supplemental Table S3 summarize these results.

The impact of NYHA class on mortality was reported in 16 studies. Based on the pooled estimates, the mortality risk of NYHA class II patients was not statistically different from NYHA class I patients (HR 1.19, 95%CI 0.92-1.55, I^2=45%). However, NYHA class III or IV patients had significantly higher mortality risk in comparison to NYHA class II or I patients (Figure 4). The subgroup analysis comparing groups with different NYHA class II, III or IV vs. I showed a significant gradient response with higher mortality risk with worsening NYHA class ($p < 0.001$). Despite this gradient in response and strong association, our confidence in estimates was very low due to the presence of risk of bias at the study level for the use of univariable analysis for variable selection, unexplained inconsistency, indirectness due to the potential measurement error associated with inter-rater variability, imprecision and some risk of reporting bias.

Left ventricular ejection fraction, reported in 18 studies, was an independent predictor of mortality. When analyzed as a continuous variable, a 10-% increase in LVEF was associated with 23% decreased mortality (HR 0.77, 95%CI 0.73-0.83, I^2=38%). When analyzed as a categorical variable, LVEF was significantly associated with

mortality and the effect estimate significantly decreased with higher cut-offs (at 20%, HR 0.51, 95%CI 0.27-0.97; at 30%, HR 0.37, 95%CI 0.21-0.66, p-value of subgroup comparison = 0.01) (Figure 5). Confidence in estimates for LVEF as a categorical variable was high but it was low when LVEF was evaluated as a categorical variable due to risk of bias and imprecision.

History of congestive HF (HR 1.67, 95%CI 1.25-2.23, I^2=84%), atrial fibrillation (HR 1.35, 95%CI 1.23-1.49, I^2=57%) and wide QRS (HR 1.33, 95%CI 1.1-1.61, I^2=51%) were also identified as independent predictors of mortality. Ischemic CMP was not associated with significantly increased mortality (HR 1.21, 95%CI 0.96-1.54, I^2=76%). Supplemental Figures S9-S11 show these results. Wide QRS warranted high confidence in estimates; history of congestive HF, atrial fibrillation and Ischemic CMP low confidence (Table 1).

Factors related to ICD therapy

Under factors related to ICD therapy, we categorized the indication for ICD (primary vs. secondary) and delivery of ICD therapy during follow up (appropriate and/or inappropriate shocks and anti-tachycardia pacing (ATP)). ICD indication was not associated with mortality (HR 1.11, 95%CI 0.93-1.32, I^2=69%) (Supplemental Figure S12). Any type of shock, including appropriate, inappropriate, any type of shock and both appropriate and inappropriate shocks, was an independent predictor. The comparison of different types of shocks showed that the mortality risk associated with appropriate shocks (HR 1.84, 95%CI 1.43-2.35, I^2=81%) was not significantly different (p>0.20) from the mortality risk associated with inappropriate shocks (HR 1.55, 95%CI 1.29-1.86,

I^2=3%), electrical storm (HR 2.4, 95%CI 1.34-4.31) or both appropriate and inappropriate shocks (HR 2.34, 95%CI 1.59-3.44, I^2=0%). These results are shown in Figure 6. The occurrence of ATP during follow up was not associated with increased mortality (high to moderate confidence).

DISCUSSION

Our results reveal that older age, poorer baseline renal function, history of COPD, diabetes and PVD, decreased LVEF and the occurrence of ICD shocks during follow up are strong predictors of mortality in ICD patients. The evidence for these variables warrants high confidence. Poorer NYHA class, history of congestive HF and atrial fibrillation are also strong predictors, however, limitations of risk of bias, inconsistency, indirectness, imprecision and publication bias reduce our confidence in the magnitude of this association. The analysis also suggest that wide QRS and non-white race are also independent predictors; the impact on mortality was, however, smaller. Ischemic CMP and male sex were not independent predictors. There was limited evaluation of some important factors, including but not limited to peak oxygen consumption (VO_2), brain natriuretic peptide (BNP) and other laboratory markers.

Some factors, such as age, renal dysfunction and LVEF, are associated with higher quality of evidence when they are treated as continuous rather than categorical variables. A potential explanation is that heterogeneity was decreased when variables were treated as continuous. This finding is likely related to the underlying distribution in the original population of each included study. For example, a study including patients

with a wide age range and using a specific cut-off of age may report a higher mortality risk than a study including a narrow range of ages and using the same cut-off. In this example, if there is a linear association, the effect of age as a continuous factor would be considered constant in different parts of the age range and would not be affected by the underlying distribution of age [12]. Based on our findings, the impact of age, renal dysfunction and LVEF on mortality when they are used as continuous variables can reliably be extrapolated to different populations; however, that may not be the case when they are treated as categorical variables.

Two recently developed prediction models on ICD patients, the PACE score [13] and the SHOCKED predictors [14], as well as the Seattle Heart Failure Survival Model (SHFM) include some of the predictors that our analysis identified as reliable strong factors. The PACE score include only four predictors (PVD, age>70years, creatinine >2mg/dL and LVEF <20%) in a continuous risk score for an individual patient from 0 to 5 and reported poor discriminatory capacity (c-statistic of 0.69). The SHOCKED predictors include seven predictors (age >75 years, NYHA class >II, atrial fibrillation, COPD, CRD, LVEF <20% and diabetes) in a continuous risk score with values from 0 to 400 to estimate 1-, 2-, 3- and 4- year survival using a nomogram and reported modest discriminatory capacity (c-statistic of 0.74). The SHFM, a model derived from non-ICD patients, has been recently tested in a mixed cohort of ICD and non-ICD patients [15] and cohorts of CRT-ICD patients [16-18] showing modest discrimination (c-statistic between 0.68-0.78). Based on the findings of this review, we anticipate that the performance of these models in subsequent cohorts may be limited due to treating predictors as

categorical rather than continuous variables and the omission of some important predictors, including ICD shocks. Researchers developing new prediction models may incorporate some of these findings to enhance performance and generalizability.

Current HF guidelines recommend the use of maximal exercise testing with measurement of respiratory gas exchange to risk-stratify patients and identify those at high-risk and who should be considered for cardiac transplantation or other advanced treatments [5]. According to a report from the UNOS (United Network Organ Sharing) database [19], 76% of heart transplant candidates between 2006 and 2009 had an ICD implanted. We identified only one study evaluating peak VO_2 [20]. They reported on 352 ICD patients that peak VO_2 was an independent mortality predictor (HR 0.98 for 1-ml/min/kg increase, 95%CI 0.97-0.99) [20]. The authors concluded that the Heart Failure Survival Score (HFSS), a score developed 20 years ago, had better discriminatory capacity than peak VO_2 alone. They also demonstrated using univariable analysis that the threshold for heart transplant listing should be reduced to 10 ml/min/kg instead of the current threshold of 12 ml/min/kg [21] in ICD patients. This demonstrates the shortcomings of the current evidence used to determine the optimal timing for instituting advanced therapies in HF patients.

Similar problems can be described with other predictors, such as BNP and other laboratory markers. As guidelines have recognized, evidence supporting the use of BNP levels in ambulatory HF patients to guide therapy and monitor disease progression is limited [3,5]. In fact, no studies were identified evaluating the role of BNP in ICD patients.

Incorporation of the identified mortality predictors in clinical practice may aid physicians in identifying high risk patients who may benefit from advanced HF therapies and end of life discussions. ICDs prolong life; however in some patients they do so at the expense of painful shocks. Approximately 30% of ICD patients experience shocks immediately before death resulting in additional psychological distress to patients and families [22]. Identification of patients nearing end of life may facilitate ICD deactivation discussions ultimately reducing the burden of unwanted ICD therapies.

Strengths and limitations

While this systematic review was conducted in a methodologically rigorous manner, it has certain limitations. Most of the patients included in this analysis were derived from observational cohort studies, enhancing the generalizability of our findings. We focused on ICD patients in order to have a closer representation of the current HF population. Most of the patients included in this analysis had a primary indication for ICD; hence, caution should be taken when extrapolating the results from this analysis to HF patients without an ICD.

We decided to perform a meta-analysis pooling results from different studies using different predictive models. The effect of a particular predictor may not be the same when different factors are included in different models. In order to overcome this limitation, we conducted and emphasized the results of sensitivity analyses using 4 or more strong predictors of mortality. This may decrease the chances of variation in effect estimates due to the use of different covariates and increase the likelihood that the pooled estimate represents the true effect.

Limited reporting of study population characteristics precludes the exploration of the impact of some factors. Specifically we could not explore the effect of use of CRT, ICD indication, specific causes of cardiomyopathy, study era or early vs. late mortality. However the study population is highly representative of clinical practice with the majority of patients having reduced EF from ischemic cardiomyopathy, on β-blockers and with an ICD for primary prevention.

CONCLUSIONS

In this systematic review and meta-analysis, older age, poorer baseline renal function, history of COPD, diabetes and PVD, decreased LVEF and the occurrence of ICD shocks during follow up were trustworthy and strong predictors of mortality in ICD patients. Ischemic cardiomyopathy and male sex were not independent predictors of mortality. These findings may help physicians to better assess prognosis in ICD patients. The use of categorical variables provided some risk of bias in the mortality risk estimates. There were a lack of studies assessing some prognostic factors, such as peak VO_2 and laboratory markers. These findings may guide researchers in the development of new models and in testing clinically important predictors.

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Table 1: Quality assessment at predictor level

Predictor	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Reporting bias**	**Large effect**	**Gradient response**	**Quality of evidence**
Age								
Continuous	Low	Low (I^2=49%, with highly overlapping confidence intervals)	No	No	No	Yes	Yes	HIGH
Categorical	Moderate *	Low - High (I^2=0% for a cut-off of 65 and 70 years but I^2= 90% for a cut-off of 75 years)	No	Yes (for a cut-off of 75 years)	No	Yes †	Yes	MODERATE
Male Sex	Low	High (I^2=70%)	No	Yes (upper bound of 95%CI >1.5 (1.59) when the pooled estimate showed no effect)	Probably no ‡	No	n/a	MODERATE
Non-white race	Low	Low (I^2=34%)	No	No	Probably no ‡	No	n/a	HIGH - MODERATE ‡

Table 1. Continued.

Predictor	Risk of bias	Inconsistency	Indirectness	Imprecision	Reporting bias	Large effect	Gradient response	Quality of evidence
Renal dysfunction								
Categorical	Moderate *	High (I^2=87% for moderate CRD and 75% for severe CRD)	Yes (due to different and unknown definitions)	Yes	Probably yes ‡	Yes †	No	VERY LOW
Continuous: GFR	Low	Low (I^2=0%)	No	No	Yes	Yes †	Probably no	HIGH
Continuous: Creatinine	Moderate *	Low (I^2=0%)	No	No	Probably no ‡	Yes †	Yes	MODERATE
Diabetes	Moderate *	Low (I^2=61%, with highly overlapping confidence intervals)	No	No	Yes	Yes †	n/a	MODERATE
COPD	Low	Low (I^2=36%)	No	No	No	Yes	n/a	HIGH
PVD	Low	Low (I^2=61%, with highly overlapping confidence intervals)	No	No	Probably yes ‡	No	n/a	HIGH - MODERATE ‡

Table 1. Continued.

Predictor	Risk of bias	Inconsistency	Indirectness	Imprecision	Reporting bias	Large effect	Gradient response	Quality of evidence
Hypertension	Moderate *	High (I^2=94%)	No	Yes	Probably no ‡	No	n/a	VERY LOW
NYHA	Moderate *	Moderate-High (I^2=45-91%)	Yes (due to the potential measurement error associated with inter-rater variability)	Yes	Probably yes ‡	Yes †	Yes	VERY LOW
LVEF								
per 10% increase	Low	Low (I^2=38%)	No	No	No	Yes	Yes	HIGH
Categorical	Moderate *	Moderate (I^2=80%)	No	Yes	Probably yes ‡	Yes †	Yes	LOW
History of congestive HF	Moderate *	Moderate (I^2=84%)	Probably yes (definition of congestive HF was not clearly stated in some studies)	No	No	Yes †	n/a	LOW

Table 1. Continued.

Predictor	Risk of bias	Inconsistency	Indirectness	Imprecision	Reporting bias	Large effect	Gradient response	Quality of evidence
Ischemic cardiomyopathy	Low	Moderate (I^2=76%)	Yes (due to the potential use of different definitions)	Yes (upper bound of 95%CI >1.5 (1.54) when the pooled estimate showed no effect)	Probably no ‡	No	n/a	LOW
Atrial fibrillation	Moderate *	Moderate (I^2=70%)	No	No	Yes	No	n/a	LOW
QRS > 120 msec	Low	Low (I^2=51%)	No	No	No	No	n/a	HIGH
Secondary vs. primary ICD	Low	Moderate (I^2=69%)	No	No	Probably yes ‡	No	n/a	MODERATE
ICD therapy (shocks)	Low	Low (except for appropriate shocks that had moderate inconsistency, I^2=0-81%)	No	No	No	Yes	n/a	HIGH - MODERATE

CRD, chronic renal disease; NYHA, New York Heart Association, LVEF, left ventricular ejection fraction; HF, heart failure; ICD, internal cardiac defibrillator.

* Most of the studies used variable selection based on statistical significance from univariable analysis.

† Large effect not considered due to risk of bias at study and/or reporting bias.

‡ Few studies that preclude adequate assessment of reporting bias.

FIGURE LEGENDS

Figure 1. Summary of pooled hazard ratio of each meta-analyzed predictor.

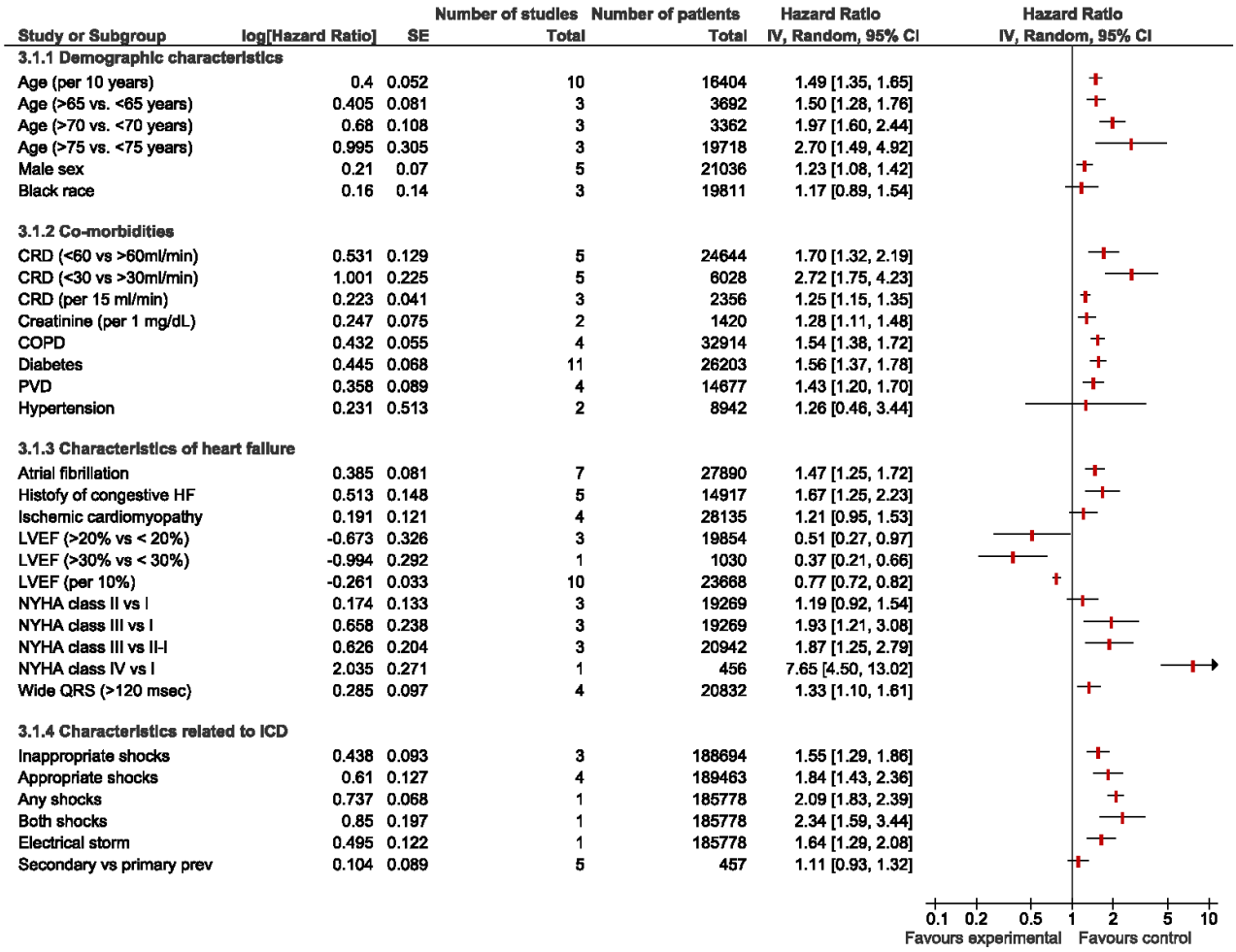


Figure 2. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of age as continuous and categorical predictor. Studies with overfitting, converted HR and those with less than 4 strong predictors included in their models were excluded. A sensitivity analysis excluding the RCT cohort of Exner 2001 showed similar results.

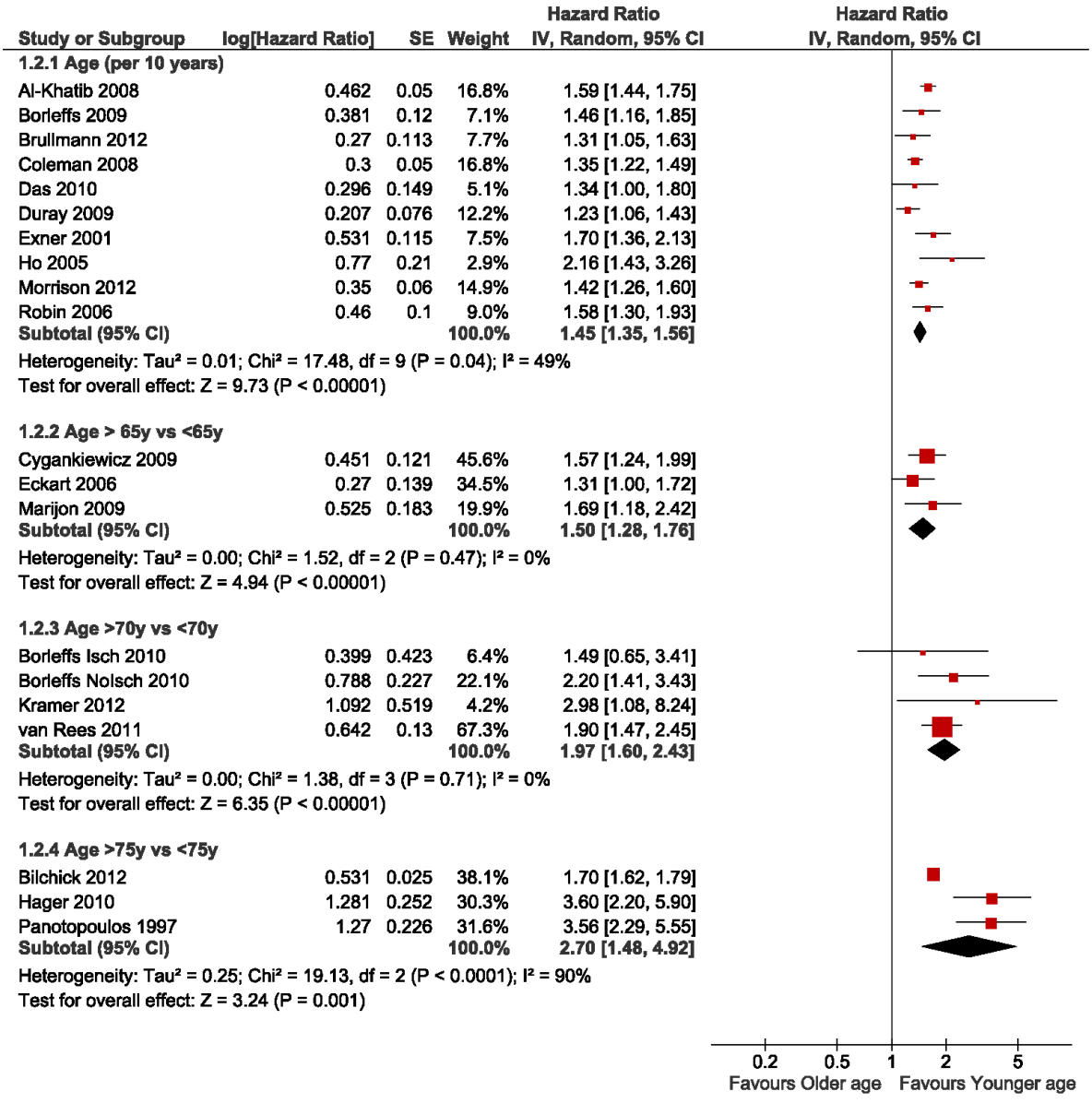


Figure 3. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of baseline renal dysfunction as continuous and categorical predictor. Studies with overfitting, converted HR and those with less than 4 strong predictors included in their models were excluded. A sensitivity analysis excluding the RCT cohort of Arshad 2011 showed similar results.

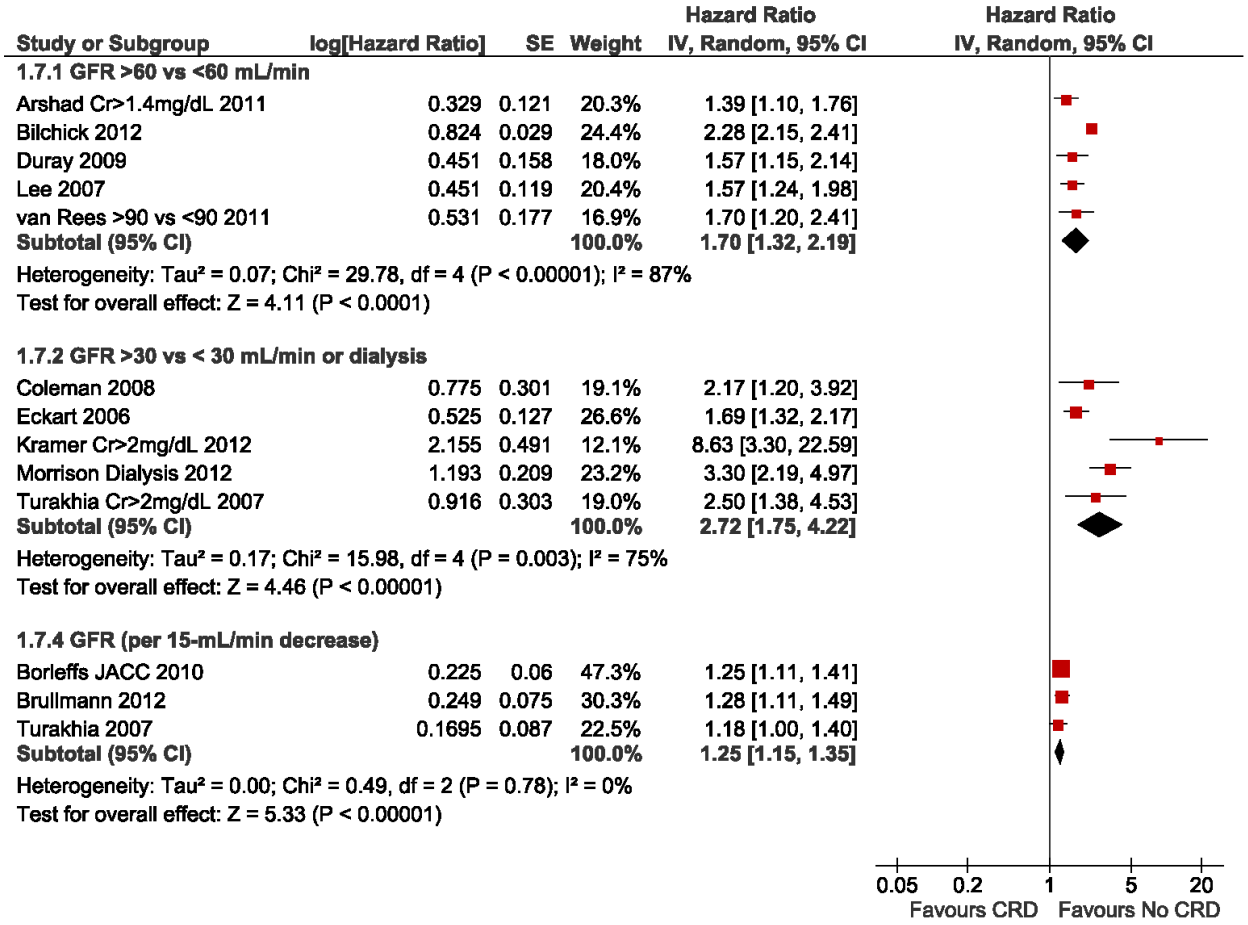


Figure 4. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of NYHA class. Studies with overfitting and those with less than 4 strong predictors included in their models were excluded. A sensitivity analysis excluding the RCT cohort of Cygankiewicz 2009 showed similar results.

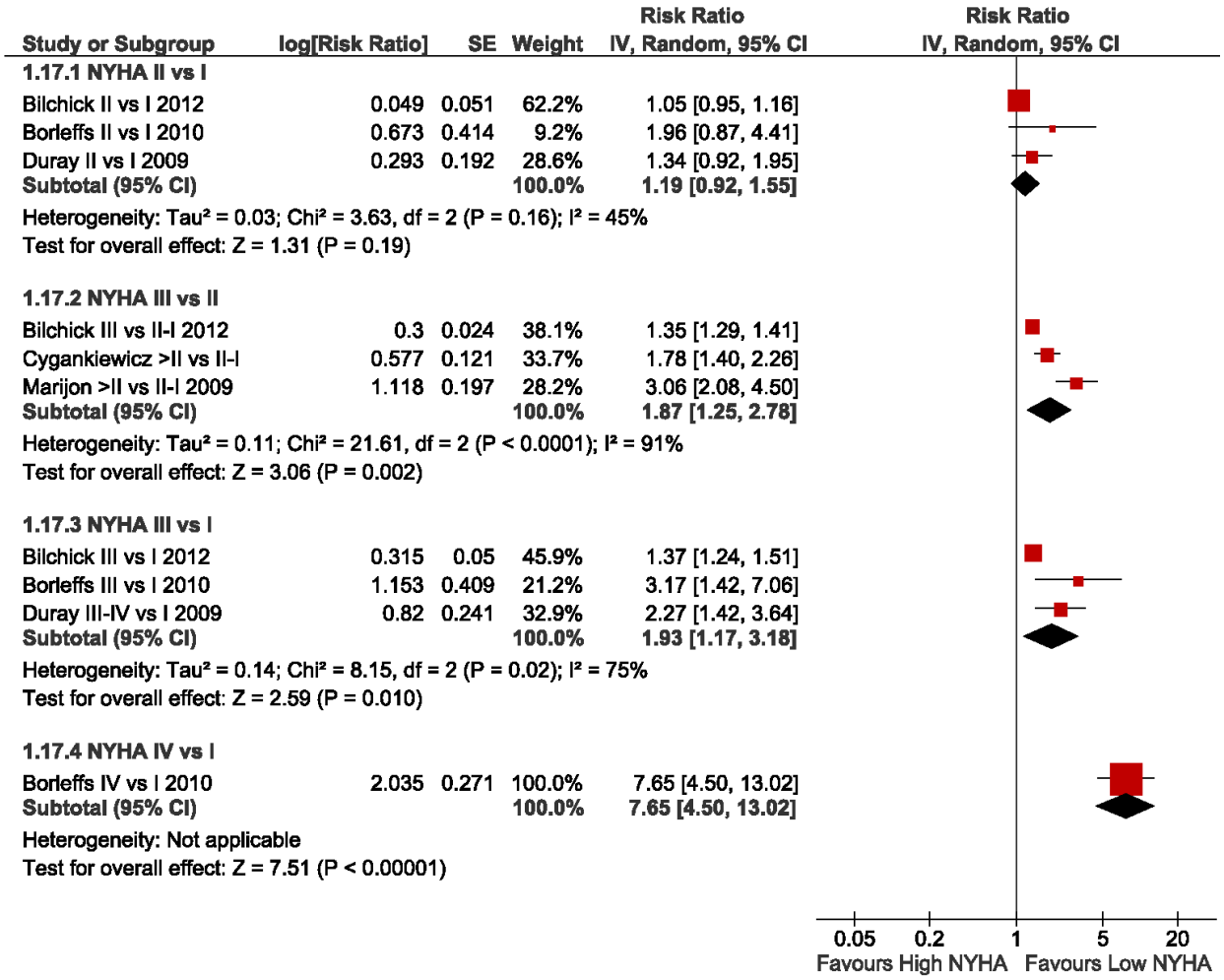


Figure 5. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of left ventricular ejection fraction as continuous and categorical predictor. Studies with overfitting and those with less than 4 strong predictors included in their models were excluded. A sensitivity analysis excluding the RCT cohorts of Arshad 2011 and Exner 2001 showed similar results.

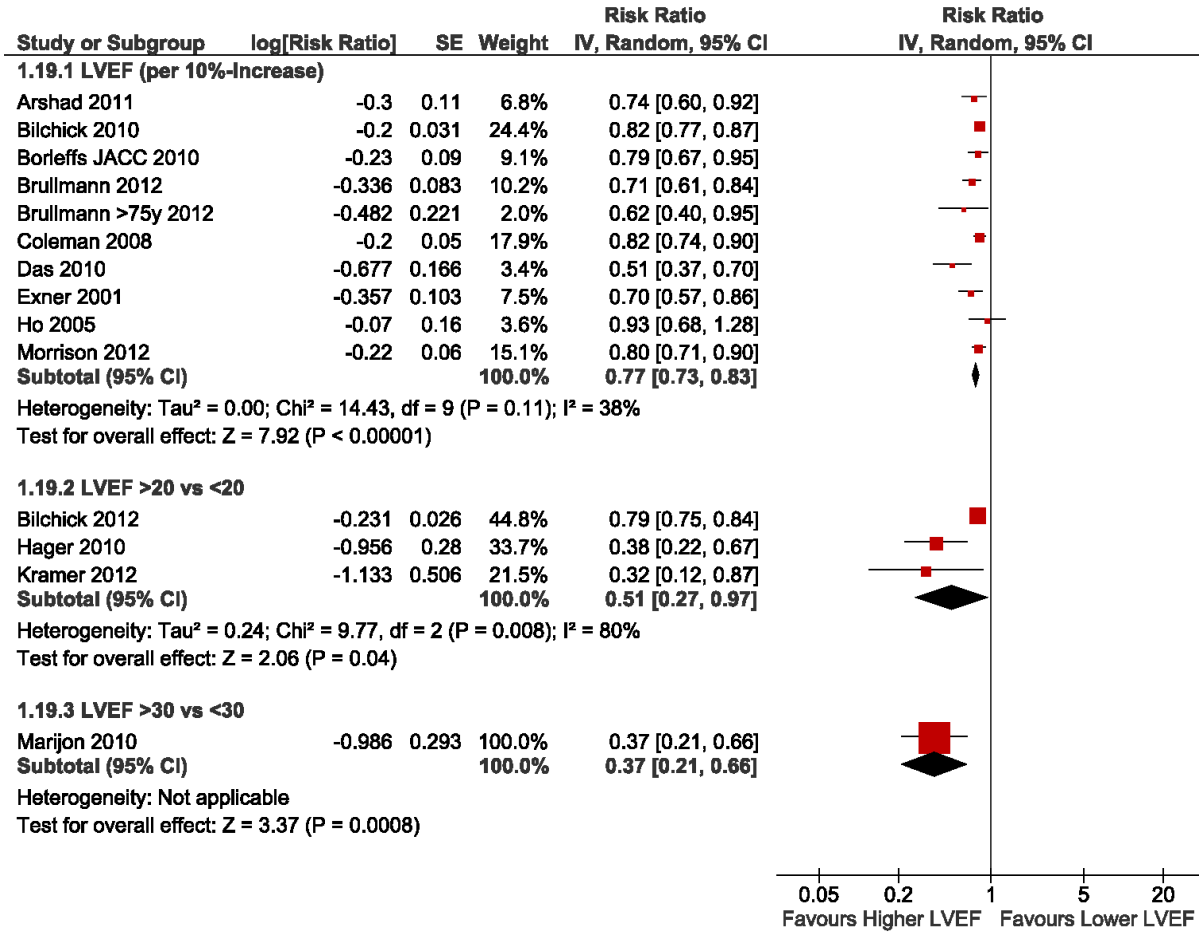
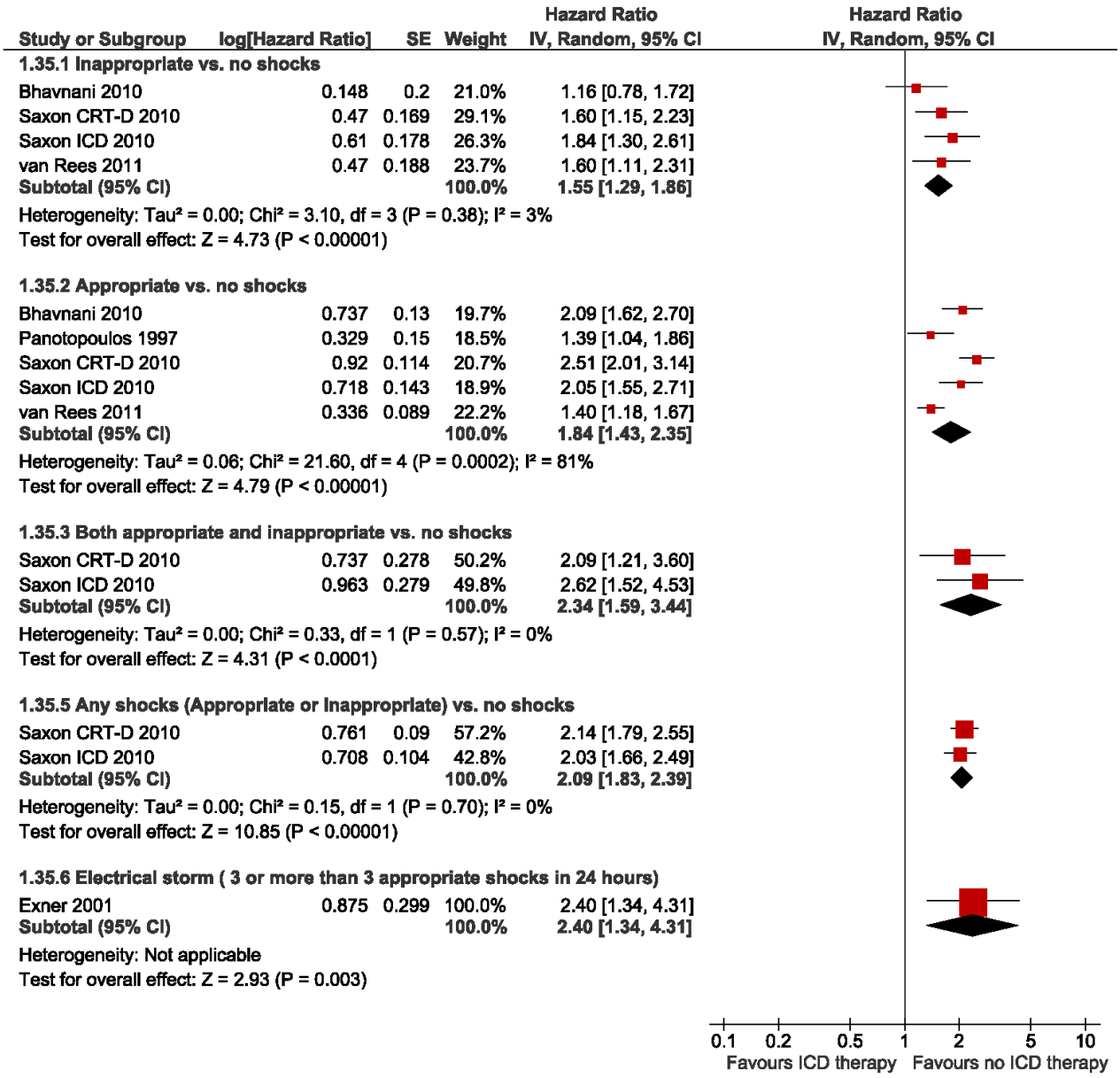


Figure 6. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of ICD therapy. Studies with overfitting and those with less than 4 strong predictors included in their models were excluded. In these studies, ICD shock was treated as time-dependent covariate or follow up started at the time of ICD shocks in patients who received an ICD shock during follow up.



SUPPLEMENTAL METHODS:

Supplemental Appendix S1: Literature search strategy and results

Date Completed: 11 May 2012

The databases searched were:

- Ovid MEDLINE(R) 1946 to May Week 1 2012
- Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations May 10, 2012
- EBM Reviews - Cochrane Database of Systematic Reviews 2005 to April 2012
- EBM Reviews - Cochrane Central Register of Controlled Trials April 2012
- Embase 1974 to 2012 May 10
- CINAHL

RESULTS & STRATEGY USED:

Database: Ovid MEDLINE(R) <1946 to May Week 1 2012>
Search Strategy:
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1 Defibrillators, Implantable/ (9500)
2 Electric Countershock/is [Instrumentation] (2416)
3 "Prostheses and Implants"/ (34005)
4 2 and 3 (353)
5 (implant: adj2 cardiac adj2 defibrillat:).mp. (306)
6 (cardioverter: adj2 defibrillat:).mp. (6631)
7 (defibrillat: adj2 pacemaker:).mp. (467)
8 (implant: adj3 defibrillat:).mp. (11567)
9 (implantable adj2 cardioverter:).mp. (5704)
10 ((heart or cardiac or cardio:) adj3 ICD).mp. (2540)
11 S-ICD:.mp. (14)
12 (subcutaneous adj2 ICD:).mp. (18)
13 1 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 (12110)
14 exp Mortality/ (247903)
15 mo.fs. (365770)
16 Survival/ (3561)
17 exp survival analysis/ (149901)
18 exp Death/ (106840)
19 (mortality or survival or death).mp. (1266793)
20 fatal outcome:.mp. (49747)
21 (fatality or fatalities).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] (15191)
22 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 (1445799)
23 13 and 22 (5113)
24 predict:.mp. (756732)
25 validat:.tw. (180066)

26 scor:.tw. (404761)
27 observ:.mp. (2029286)
28 evaluation.mp. (1000618)
29 exp Prognosis/ (930163)
30 exp risk/ (713365)
31 risk:.mp. (1306452)
32 (prognos: adj2 factor:).mp. (50696)
33 (associat: adj2 factor:).mp. (73840)
34 or/24-33 (5162296)
35 23 and 34 (3681)

Database: Ovid MEDLINE(R) In-Process & Other Non-Indexed Citations <May 10, 2012>
Search Strategy:
---

1 (implant: adj2 cardiac adj2 defibrillat:).mp. (26)
2 (cardioverter: adj2 defibrillat:).mp. (406)
3 (defibrillat: adj2 pacemaker:).mp. (32)
4 (implant: adj3 defibrillat:).mp. (455)
5 (implantable adj2 cardioverter:).mp. (344)
6 ((heart or cardiac or cardio:) adj3 ICD).mp. (170)
7 S-ICD:.mp. (3)
8 (subcutaneous adj2 ICD:).mp. (4)
9 or/1-8 (508)
10 (mortality or survival or death).mp. (51731)
11 fatal outcome:.mp. (229)
12 (fatality or fatalities).mp. [mp=title, abstract, original title, name of substance word, subject heading word, protocol supplementary concept, rare disease supplementary concept, unique identifier] (656)
13 10 or 11 or 12 (52287)
14 9 and 13 (205)
15 predict:.mp. (63574)
16 validat:.tw. (17084)
17 scor:.tw. (24469)
18 observ:.mp. (150453)
19 evaluation.mp. (34744)
20 prognosis.mp. (8818)
21 risk:.mp. (56396)
22 (prognos: adj2 factor:).mp. (2282)
23 (associat: adj2 factor:).mp. (4325)
24 or/15-23 (295231)
25 14 and 24 (142)

Database: EBM Reviews - Cochrane Database of Systematic Reviews <2005 to April 2012>
Search Strategy:
---

1 (implant: adj2 cardiac adj2 defibrillat:).mp. (4)
2 (cardioverter: adj2 defibrillat:).mp. (5)
3 (defibrillat: adj2 pacemaker:).mp. (7)

4 (implant: adj3 defibrillat:).mp. (22)
5 (implantable adj2 cardioverter:).mp. (5)
6 ((heart or cardiac or cardio:) adj3 ICD).mp. (6)
7 S-ICD:.mp. (0)
8 (subcutaneous adj2 ICD:).mp. (0)
9 or/1-8 (27)

Database: EBM Reviews - Cochrane Central Register of Controlled Trials <April 2012>
Search Strategy:
---
1 Defibrillators, Implantable/ (582)
2 Electric Countershock/is [Instrumentation] (0)
3 "Prostheses and Implants"/ (411)
4 2 and 3 (0)
5 (implant: adj2 cardiac adj2 defibrillat:).mp. (32)
6 (cardioverter: adj2 defibrillat:).mp. (498)
7 (defibrillat: adj2 pacemaker:).mp. (52)
8 (implant: adj3 defibrillat:).mp. (746)
9 (implantable adj2 cardioverter:).mp. (434)
10 ((heart or cardiac or cardio:) adj3 ICD).mp. (267)
11 S-ICD:.mp. (2)
12 (subcutaneous adj2 ICD:).mp. (0)
13 1 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 (766)
14 exp Mortality/ (8081)
15 mo.fs. (16331)
16 Survival/ (80)
17 exp survival analysis/ (11494)
18 exp Death/ (1251)
19 (mortality or survival or death).mp. (48373)
20 fatal outcome:.mp. (91)
21 (fatality or fatalities).mp. [mp=title, original title, abstract, mesh headings, heading words, keyword] (454)
22 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 (51644)
23 13 and 22 (365)
24 predict:.mp. (29608)
25 validat:.tw. (6461)
26 scor:.tw. (62831)
27 observ:.mp. (96658)
28 evaluation.mp. (57346)
29 exp Prognosis/ (80086)
30 exp risk/ (21318)
31 risk:.mp. (60238)
32 (prognos: adj2 factor:).mp. (2629)
33 (associat: adj2 factor:).mp. (2461)
34 or/24-33 (278061)
35 23 and 34 (302)

Database: Embase <1974 to 2012 May 10>
Search Strategy:
---
1 defibrillator/ (18682)
2 (implant: adj2 cardiac adj2 defibrillat:).mp. (513)
3 (cardioverter: adj2 defibrillat:).mp. (9693)
4 (defibrillat: adj2 pacemaker:).mp. (1911)
5 (implant: adj3 defibrillat:).mp. (12651)
6 (implantable adj2 cardioverter:).mp. (8538)
7 ((heart of cardiac or cardio:) adj3 ICD).mp. (3689)
8 S-ICD:.mp. (37)
9 (subcutaneous adj2 ICD:).mp. (45)
10 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 (21402)
11 exp mortality/ (540659)
12 exp survival/ (456231)
13 exp death/ (376126)
14 (mortality or survival or death).mp. (1804949)
15 fatal outcome:.mp. (7876)
16 (fatality or fatalities).mp. (102531)
17 11 or 12 or 13 or 14 or 15 or 16 (1941540)
18 10 and 17 (9774)
19 predict:.mp. (982830)
20 validat:.tw. (256212)
21 scor:.tw. (562176)
22 observ:.mp. (2607401)
23 evaluation.mp. (1127632)
24 prognosis/ (386793)
25 exp risk/ (1173786)
26 risk:.mp. (1851822)
27 (prognos: adj2 factor:).mp. (72791)
28 (associat: adj2 factor:).mp. (96204)
29 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 (6257385)
30 18 and 29 (6195)
31 limit 30 to embase (5491)

CINAHL Search Strategy Friday, May 11, 2012 12:35:40 PM

#	Query	Limiters/Expanders	Last Run Via	Results
S31	S19 and S30	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	1027
S30	S20 or S21 or S22 or S23 or S24 or S25 or S26 or S27 or S28 or S29	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search	726325

			Database - CINAHL	
S29	TX associat* N2 factor*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	16815
S28	TX prognos* N2 factor*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	3158
S27	TX risk*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	282632
S26	(MH "Risk Assessment") OR (MH "Risk Factors") OR (MH "Relative Risk") OR (MH "Attributable Risk")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	88559
S25	(MH "Prognosis+")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	118711
S24	TX evaluation	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	262466
S23	TX observ*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	111882
S22	TI scor* or AB scor*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	76612
S21	TI validat* or AB validat*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	23571
S20	TX predict*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	96169
S19	S10 and S18	Search modes -	Interface - EBSCOhost	1611

		Boolean/Phrase	Search Screen - Advanced Search Database - CINAHL	
S18	S11 or S12 or S13 or S14 or S15 or S16 or S17	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	172768
S17	TX fatal outcome* or TX fatality or TX fatalities	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	4740
S16	(MH "Fatal Outcome")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	2298
S15	TX mortality or TX survival or TX death	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	158566
S14	(MH "Death+")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	21921
S13	(MH "Survival Analysis+")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	27036
S12	MW MO	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	40940
S11	(MH "Mortality+")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	26992
S10	S1 or S2 or S3 or S4 or S5 or S6 or S7 or S8 or S9	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	4625
S9	TX subcutaneous N2 ICD*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search	9

			Database - CINAHL	
S8	TX S-ICD*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	3
S7	TX heart N3 ICD or TX cardiac N3 ICD or TX cardio* N3 ICD	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	770
S6	TX implantable N2 cardioverter*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	2081
S5	TX implant* N3 defibrillat*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	4491
S4	TX defibrillat* N2 pacemaker*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	198
S3	TX cardioverter* N2 defibrillat*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	2352
S2	TX implant* N2 cardiac N2 defibrillat*	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	141
S1	(MH "Defibrillators, Implantable")	Search modes - Boolean/Phrase	Interface - EBSCOhost Search Screen - Advanced Search Database - CINAHL	

Supplemental Appendix S2: Study eligibility form[1]

Reviewer:	JB	CA	NN

Article ID:			
RefWorks #:	Author:	Journal:	Year:

Population:		
• All ICD patients	**YES**	**NO**
• Adults (≥ 18 years old)		

Predictor[2]:		
• Any mortality predictor	**YES**	**NO**

Adjusted Analysis[3]:		
• Multivariate analysis with:		
• ≥ 3 predictors *or*	**YES**	**NO**
• 2 predictors, if one is a score		

Outcomes reported:		
• Mortality (with >30 deaths)	**YES**	**NO**

Type of article[4]:		
• Cohort study or	**YES**	**NO**
• RCT		

Duplicated population:		
• If duplicated, does this study provide new information?	**YES**	**NO**

Study inclusion:	
• All the answers are YES	**INCLUDE**
• Any answer is NO	**EXCLUDE**

References:

[1] If any response to the above questions is unclear, mark YES

[2] Consider YES in any type of predictor, including but not limited to clinical characteristics, laboratory values, test results and any other clinical event, such as hospital admissions, ICD shocks, etcetera.

[3] Consider NO if the study used any other type of adjustment for potential confounders, including matched design or stratification.

[4] Consider YES if it is a post-hoc analysis of an RCT.

Supplemental Appendix S3. Items considered in the assessment of risk of bias at study level

Item	Description
Patient selection	Consecutive or random patient selection was deemed as high quality. Other type of patient selection, such as convenient, was considered as low quality.
Study design	Prospective cohort studies were deemed as high quality because of high probability of optimal documentation of predictors and outcomes. Retrospective cohort studies with prospectively collected data, including a cohort of individuals that participated in a randomised therapeutic trial, were considered as providing some risk of bias and retrospective cohort studies were considered as low quality.
Loss of follow up	Studies reporting more than 5% of loss of follow were considered as low quality; between 1% and 5% of loss of follow up moderate and less than 1% as high quality. Studies that did not report the frequency of loss of follow up were considered as low quality.
Selection of the predictors	In studies where predictors were chosen according to clinical importance and using full models were deemed as high quality. In studies were candidates predictors were chosen based on statistical significance in univariable analysis and then selected using backward elimination or kept into the final model (full model) were considered as moderate quality and studies using forward elimination and only retaining predictors that were significantly associated with the outcome were considered as low quality.
Missing values	Studies that used some statistical techniques to impute missing values, preferably multiple imputation, were considered as high quality. Studies not reporting the frequency of missing values and studies analyzing individuals with only completely observed data were considered as low quality.
Conformity to linearity for continuous predictors	In the case of continuous predictors (i.e. age), studies that did not report determining the impact of the continuous predictor separately in zones of ranked data or mentioned that conformity to a linear association with the outcome was addressed were considered as presenting some risk of bias.

	Studies reporting that a continuous predictor showed linear relationship with the outcome or using more than one category when linear gradient was not present were deemed as high quality.
Over-fitting	Studies having model overfitting were deemed as low quality. Risk estimates may be unreliable if the multivariable model includes too many independent variables and too few outcome events, they may represent spurious associations or the effects may be estimated with low precision. According to Peduzzi et al [1], we categorized the articles with a ratio of < 10:1 (10 outcome events for each single explanatory variable in the final model) as an over-fitted model.
Analysis of statistical model assumptions	Violation of model assumptions, such as the proportional hazards assumption in the case of Cox method, may lead to unreliable effect estimates. Studies that did not state exploring model assumptions or reporting that assumptions were not held in the final proposed model were coded as low quality.
Internal or external validation	Internal validation (using bootstrapping techniques) or external validation (in an independent cohort) can quantify the model's potential for false associations. Studies that did not validate its results were considered at some risk of bias. Studies validating its results were considered as high quality.

Reference

1. Peduzzi P, Concato J, Feinsten AR, Holford TR. Importance of events per independent variable in proportional hazards regression analysis II. Accuracy and precision of regression estimates. J Clin Epidemiol 1995;48:1503-10.

Supplemental Appendix S4. Assessment of the confidence in the evidence.

Factors considered in the assessment of confidence in the evidence. The confidence starts as high and may be downgraded or upgraded due to the presence of the following factors:

Factors	Description
Factors that decrease quality of the evidence	
Risk of bias	Confidence in the evidence decreases if studies have major limitations that may bias (underestimate or overestimate) the association between predictors and mortality. These limitations include convenient (and not random or consecutive) selection of patients, retrospective data collection, overfitting, selection of variables based on statistical significance from univariable analysis, significant loss of follow up (>5%).
Inconsistency	Broadly inconsistent estimates of the predictor effect on mortality reflects heterogeneity or variability in results suggesting the presence of true differences in the underlying association between the predictor and mortality. Unexplained inconsistency decreases confidence in the evidence. The assessment of inconsistency was based on the I^2 static and expert judgement. We analyzed study consistency using the I^2 statistic, which represents an estimate of the proportion of total variation that is likely to represent between study variation or heterogeneity across studies. The presence of heterogeneity was judged based on Cochrane's recommendations [8]: 0-40% might not represent important heterogeneity, 30-60% may represent moderate, 50-90% may represent substantial and 75-100% may represent considerable heterogeneity; the overlapping boundaries acknowledge the judgment that is required in the interpretation; therefore, when results fall in or about the overlapping sections of categories, we considered the likelihood of under- or over-powered estimates and the clinical significance of the extent of variability.
Imprecision	Imprecise estimates are characterized by wide confidence intervals. These are more common in studies including few events and may also decrease our confidence in the evidence especially if they include important differences in both directions (negative vs. positive association).

Supplemental Appendix S4. Continued.

Factors	Description
Indirectness	Indirectness in the evidence includes differences in the population (i.e. post-hoc analysis of an RCT cohort versus observational "real-world" cohort), definition of the predictor (i.e. differences in the measurement of renal dysfunction or unknown definition) and definition of the outcomes (i.e. all cause mortality or cardiovascular mortality). Confidence in the evidence decreases if the presence of differences may bias the estimate of the association between the predictor and mortality.
Publication bias or selective reporting bias	In some instances, investigators do report the association of factors that were not significantly associated with mortality. The presence of selective reporting bias decreases the confidence in the evidence since estimates of the association between a predictor and mortality may be overestimated. Presence of reporting bias was assessed using funnel plots.
Factors that increase quality of the evidence	
Large effect	In the absence of risk of bias at study level and publication bias, confidence in the evidence increases if studies describe a large or very large association between a predictor and mortality. The magnitude of the effect was judged as large considering the point estimate and its confidence interval. In case of categorical variables, the presence of a HR of 1.5 and a ratio of the natural logarithmic function of the HR and its standard error >3 was considered as indicating a strong association between the factor and mortality. In the case of a continuous variable, a ratio of the natural logarithmic function of the HR and its standard error >3 was considered as indicating a strong association.
Presence of gradient response	The presence of larger association between a predictor and mortality with increasing severity of the predictor (i.e. older age, more severe renal dysfunction) increases the confidence in the evidence. Gradient response was tested using subgroup analysis. A p- value ≤ 0.05 was considering as statistically significant suggesting the presence of gradient response.

SUPPLEMENTAL TABLES

Supplemental Table S1. Characteristics of the studies included

Study	Population												Outcomes			Predictors *
	Source	**Study design**	**Time frame**	**N**	**Age Mean ± SD**	**% male**	**LVEF Mean ± SD**	**% ischemic CMP**	**QRS Mean ± SD**	**% β-blocker**	**% CRT**	**% primary indication**	**Follow-up (years) Mean± SD**	**n**	**1-year survival**	
Agarwal 2007	Single center Pittsburgh - US	RC	nr	80	64 ± 12	80	33 ± 14	63	127 ± 32	46	nr	0	4.7 ± 2.3	40	93.8%	Age, creatinine, QRS
Al-Kahit 2008	Medicare database US	RC	2002-2005	8581	75 ± 6	77	n.r.	94	n.r.	n.r.	29	n.r.	n.r. (maximum of 2.7)	1060	87.6%	Age, sex, race, CRD, COPD, HTN, DM, PVD, *cancer*, *stroke*, *dementia*, CRT, congestive HF, HF etiology, *year ICD implant*, *physician qualities*
Arshad 2011	MADIT-CRT trial US	RCT	2004-2008	1820	64 ± 11	75	24 ± 5	55	158 ± 19	93	60	93	2.4	54	98%	Race, creatinine, LVEF, NYHA, *6'WT*, A Fib, CRT

Study	Population												Outcomes			Predictors *
	Source	**Study design**	**Time frame**	**N**	**Age Mean ± SD**	**% male**	**LVEF Mean ± SD**	**% ischemic CMP**	**QRS Mean ± SD**	**% β-blocker**	**% CRT**	**% primary indication**	**Follow-up (years) Mean± SD**	**n**	**1-year survival**	
Barsheshet 2011	MADIT II trial US	RCT	1997-2001	567	64 ± 10	80	24 ± 5	100	123 ± 35	65	0	100	7.4	n.r.	94.9%	*RV pacing*
Barsheshet 2011	MADIT II trial US	RCT	1997-2001	720	64 ± 10	85	24 ± 5	100	124 ± 35	64	0	100	n.r. (maximum 8 years)	99	94.9%	CABG
Bhavnani 2010	Single center Connecticut - US	RC	1997-2007	1372	59 ± 12	79	23 ± 9	53	n.r.	72	24	61	2.2 ± 2.2	357	86.3%	ICD shocks
Bilchick 2010	Medicare database US	RC-P	2005-2006	14946	73 ± 11	73	23 ± 6	69	157 ± 25	79	100	79	3.3	5557	87.4%	Age, sex, smoking, HTN, DM, *stroke*, *dementia*, CABG, CRT, congestive HF, HF etiology, LVEF, NYHA, QRS, *BP*, *HR*, A Fib, ACEI, BB, diuretics, amiodarone, ICD indication, *physician qualities*

Supplemental Table S1. Continued.

Study	Population												Outcomes			Predictors *
	Source	**Study design**	**Time frame**	**N**	**Age Mean ± SD**	**% male**	**LVEF Mean ± SD**	**% ischemic CMP**	**QRS Mean ± SD**	**% β-blocker**	**% CRT**	**% primary indication**	**Follow-up (years) Mean± SD**	**n**	**1-year survival**	
Bilchick 2012	Medicare database US	RC-P	2005-2007	17991	73 ± 11	88	23 ± 6	59	n.r.	80	n.r.	100	4.4	9741	87%	Age, sex, race, CRD, COPD, HTN, DM, *cancer*, *depression*, congestive HF, HF etiology, LVEF, NYHA, QRS, *BP*, *HR*, A Fib, CRT, ACEI, BB, digoxin, diuretics, amiodarone
Blatt 2008	SCD-HeFT	RCT	1997-n.r.	717	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	n.r.	100	n.r.	146	94%	*Defibrillation threshold*
Blendea 2009	Single center US	RC	nr	174	68 ± 12	82	29 ± 11	82	nr	nr	nr	nr	3.5	56	83%	*Troponins*
Bocker 1998	Single center Germany	RC	1986-196	603	57 ± 13	77	44 ± 18	59	n.r.	n.r.	n.r.	7	2.1 ± 1.8	71	93.7%	LVEF, NYHA

Study	Population												Outcomes			Predictors *
	Source	Study design	Time frame	N	Age Mean ± SD	% male	LVEF Mean ± SD	% ischemic CMP	QRS Mean ± SD	% β-blocker	% CRT	% primary indication	Follow-up (years) Mean± SD	n	1-year survival	
Boriani 2012	INSync ICD registry Italy	RC-P	n.r	659	66 ± 9	90	26 ± 7	68	163 ± 31	53	100	43	1.2	66	95%	Age, BB
Borleffs 2009	LOHCAT registry Netherlands	RC	1996-2007	456	65 ± 10	86	35 ± 14	100	119 ± 30	46	n.r.	0	4.5 ± 2.9	100	96%	Age, DM, QRS, A Fib, creatinine, diuretics, statins
Borleffs 2010	LOHCAT registry Netherlands	RC	1996-2007	913	62 ± 11	80	32 ± 14	61	127 ± 35	76	61	81	2.3 ± 1.1	117	94%	Age, sex, creatinine, GFR, LVEF, NYHA, QRS, A Fib, BB
Brodine 2005	MADIT II trial US	RCT	1997-2001	720	64 ± 10	85	23 ± 5	100	124 ± 35	64	0	100	1.8	117	90.7%	BB
Brullman 2012	Multicenter Europe	RC	1987-2009	936	63	85	30 ± 12	58	n.r.	n.r.	25	42	3.6 ± 2.5	214	92.3%	Age, sex, GFR, LVEF, QRS
Bunch 2009	INSTRINSIC RV trial Multicenter	RCT	2003-2004	1530	65 ± 12	81	n.r.	58	n.r.	76	0	n.r.	1	71	n.r.	A Fib
Chow 2008	Multicenter US	PC	2003-2007	575	65 ± 11	84	24 ± 5	100	119 ± 30	87	23	100	2.1 ± 1	59	97%	*Microvolt T-wave alternans*

Supplemental Table S1. Continued.

Study	Population												Outcomes			Predictors *
	Source	Study design	Time frame	N	Age Mean ± SD	% male	LVEF Mean ± SD	% ischemic CMP	QRS Mean ± SD	% β-blocker	% CRT	% primary indication	Follow-up (years) Mean± SD	n	1-year survival	
Coleman 2008	Single center Connecticut - US	RC	1997-2007	1204	66 ± 12	79	23 ± 9	72	n.r.	81	28	60	2.6 ± 2.6	314	99%	Age, sex, CRD, COPD, DM, *PH*, LVEF, ACEI, statins, aldosterone, CRT, amiodarone
Cuculich 2007	Single center US	RC	1999 - 2005	229	67	84	25	60	nr	86	23	100	1.5 ± 1.2	33	90.9%	CRD
Cygankiewicz 2009	MADIT II trial US	RCT	1997-2001	655	64 ± 10	93	28 ± 5	100	136 ± 35	64	0	100	5.25	294	91.5%	Age, DM, *BUN*, NYHA, A Fib
Das 2010	Single center Indianapolis - US	RC	2002-2005	361	63 ± 11	91	27	68	125	61	n.r.	55	1.3 ± 0.9	54	93.6%	Age, DM, HTN, creatinine, QRS, ICD indication
Daubert 2008	MADIT II trial US	RCT	1997-2001	719	64 ± 10	85	23 ± 5	100	124 ± 35	65	0	100	1.3 ± 1	93	91%	Congestive HF, *HF admissions*, *BUN*, BB, ICD shocks

Supplemental Table S1. Continued.

Study	Population												Outcomes			Predictors *
	Source	Study design	Time frame	N	Age Mean ± SD	% male	LVEF Mean ± SD	% ischemic CMP	QRS Mean ± SD	% β-blocker	% CRT	% primary indication	Follow-up (years) Mean± SD	n	1-year survival	
Desai 2010	Single center US	RC	nr	549	74 ± 10	79	29 ± 7	59	118 ± 19	68	38	nr	3.4 ± 1.8	63	n.r.	Age, HTN, A Fib, ACEI, digoxin, statins, *RV pacing*
Desai 2010	Single center US	RC	nr	549	74 ± 10	79	29 ± 7	59	118 ± 19	68	38	nr	3.4 ± 1.8	62	n.r.	Age, DM, HTN, A Fib, ACEI, statins, CRT, *RV pacing*
Dickinson 2007	SCD-HeFT	RCT	1997-n.r.	829	60	75	24	52	n.r.	n.r.	n.r.	100	3.8	214	94%	Statins
Dubner 2005	Multicenter Latin America	PC	1995-2004	739	60 ± 13	75	38 ± 14	49	n.r.	10	0	0	2.25 ± 2	130	94.8%	Age, sex, NHYA, LVEF, HF etiology
Duray 2009	Single center Germany	RC	1995-2001	822	63 ± 11	80	34 ± 13	71	n.r.	74	6	35	3.6 ± 2.5	225	92.4%	Age, CRD, NYHA, HF etiology, LVEF, digoxin, diuretics, statins, amiodarone
Eckart 2006	Single center MDR database - US	RC	2002-2004	741	64 ± 14	80	n.r.	84	n.r.	n.r.	n.r.	94	4.4 ± 3.7	107*	94.5%	Age, CRD, DM, congestive HF, HF etiology

Supplemental Table S1. Continued.

Study	Population												Outcomes			Predictors *
	Source	**Study design**	**Time frame**	**N**	**Age Mean ± SD**	**% male**	**LVEF Mean ± SD**	**% ischemic CMP**	**QRS Mean ± SD**	**% β-blocker**	**% CRT**	**% primary indication**	**Follow-up (years) Mean± SD**	**n**	**1-year survival**	
Exner 1999	AVID trial US and Canada	RCT	1993-1998	507	64 ± 11	78	32 ± 13	67	n.r.	41	n.r.	0	2.6 ± 1	103	n.r.	Age, race, BB, ACEI, amiodarone
Exner 2001	AVID trial US and Canada	RCT	1993-1998	507	64 ± 11	78	32 ± 13	67	n.r.	41	n.r.	0	2.6 ± 1	103	n.r.	Age, sex, DM, congestive HF, LVEF
Gatzoulis 2005	Single center Greece	PC	1997-2004	169	60 ± 12	84	34 ± 14	60	n.r.	100	n.r.	11	2.8 ± 2.2	36	98%	NYHA, ICD shocks
Goda 2011	Single center Sweden	RC	1993 - 2008	352	54 ± 11	73	20 ± 7	45	nr	77	31	nr	2.6 ± 2.5	153	n.r.	Age, sex, *BMI*, *sodium*, LVEF, HF etiology, *peak VO_2*, *HR*, *BP*, QRS, CRT
Gold 2007	LESS trial US	RCT	n.r.	627	66 ± 12	77	35 ± 14	n.r.	n.r.	n.r.	38	n.r.	2 ± 0.3	233	90.5%	*ICD pocket location*
Hager 2012	2 US centers	RC	2000-2006	958	67	n.r.	27 ± 8	75	n.r.	n.r.	42	100	n.r. (min of 1 year)	73	92.5%	Age, LVEF, DM, CRD, PVD
Ho 2005	Single center Loma Linda - US	RC	n.r.	360	62 ± 13	50	33 ± 17	68	n.r.	46	n.r.	n.r.	4.4 ± 3.7	68	96%	Age, LVEF, A Fib, congestive HF, HF etiology, digoxin, BB, amiodarone

Supplemental Table S1. Continued.

Study	Population												Outcomes			Predictors *
	Source	**Study design**	**Time frame**	**N**	**Age Mean ± SD**	**% male**	**LVEF Mean ± SD**	**% ischemic CMP**	**QRS Mean ± SD**	**% β-blocker**	**% CRT**	**% primary indication**	**Follow-up (years) Mean± SD**	**n**	**1-year survival**	
Koller 2008	Single center Switzerland	PC	1995 - 2006	442	64 ± 12	89	30 ± 7	76	nr	85	16	41	3.6	73	97%	Age, LVEF, ACEI, BB, diuretics, ICD indication, *year ICD implant*
Koplan 2006	Single center US	RC	1995 - 2003	348	70 ± 3	83	30 ± 12	80	nr	nr	12	42	3.3 ± 2.2	170	88.5%	Sex, CRD, DM, HF etiology, LVEF, QRS, CRT, ICD indication
Kramer 2012	Multicenter US	RC	2001-2008	905	66 ± 14	78	31 ± 15	59	n.r.	n.r.	31	76	3.2 ± 1.8	125	96%	Age, creatinine, PVD, LVEF
Ladwiq 2008	LICAD study Germany	CC PC	1998-2003	147	62 ± 12	85	n.r.	n.r.	n.r.	59	n.r.	n.r.	5.1 ± 2.2	45	96.5%	*Post-traumatic stress disorder*
Larsen 2011	Single center Wisconsin - US	RC	1983 - 1995	425	64 ± 10	99	33 ± 11	90	118 ± 20	84	11	41	3.4 ± 2	171	92.7%	ICD shocks, *Seattle HF model*
Lee 2007	CIHI database Canada	RC-P	1997-2003	2467	62 ± 13	79	n.r.	71	n.r.	n.r.	n.r.	16	n.r. (minimum of 2 years)	346	92.2%	Age, CDR, DM, PVD, COPD, congestive HF, ICD indication

Supplemental Table S1. Continued.

Study	Population												Outcomes			Predictors *
	Source	Study design	Time frame	N	Age Mean ± SD	% male	LVEF Mean ± SD	% ischemic CMP	QRS Mean ± SD	% β-blocker	% CRT	% primary indication	Follow-up (years) Mean± SD	n	1-year survival	
Lee 2010	Ontario ICD database Canada	RC-P	2007-2009	3340	64 ± 13	79	30 ± 10	67	130 ± 36	85	24	70	n.r. (maximum 6 months)	65	n.r.	*Major and minor complications after ICD implant*
Levine 1991	Single center US	RC	1980 - 1987	105	57 ± 13	nr	35 ± 16	nr	nr	nr	nr	0	nr (maximum 7.2 years)	82	n.r.	*Type of ICD, other concomitant surgical procedures*
Levy 2008	Single center US	RC	1992 - 2004	346	65 ± 3	80	30 ± 15	74	nr	nr	nr	nr	3.5	67	93.5%	CRD, DM, LVEF
MacFadden 2012	Ontario ICD database Canada	RC-P	2007-2010	6021	65 ± 12	79	29 ± 11	n.r.	129 ± 36	n.r.	n.r.	72	n.r.	141	n.r.	Sex
Marijon 2009	EVADEF database France	RC-P	2001-2004	2296	60 ± 15	86	39 ± 16	57	117 ± 35	62	8	n.r.	1.7 ± 0.5	156 †	92.8%	Age, HTN, A Fib, NYHA, LVEF, BB
Marijon 2010	EVADEF database France	RC-P	2001-2003	1030	63 ± 12	89	36 ± 13	70	117 ± 35	70	53	14	1.7 ± 0.5	52 †	94%	LVEF, BB, diuretics

Supplemental Table S1. Continued.

Study	Population												Outcomes			Predictors *
	Source	**Study design**	**Time frame**	**N**	**Age Mean ± SD**	**% male**	**LVEF Mean ± SD**	**% ischemic CMP**	**QRS Mean ± SD**	**% β-blocker**	**% CRT**	**% primary indication**	**Follow-up (years) Mean± SD**	**n**	**1-year survival**	
Mitchell 2003	AVID trial US and Canada	RCT	1993-1998	362	65 ± 10	83	32 ± 12	n.r.	n.r.	63	n.r.	0	2.3 ± 1	91	88%	Statins
Morrison 2012	Multicenter US	RC	2001-2008	2671	65 ± 14	77	31 ± 15	59	n.r.	n.r.	31	76	3.2 ± 1.8	398	97%	Age, CRD, COPD, PVD, A Fib, congestive HF, LVEF, ICD indication, *atrial lead, number of ICD generator replacement*
Moss 2004	MADIT II trial US	RCT	1997-2001	720	64 ± 10	85	23 ± 5	100	124 ± 35	64	0	100	1.8 ± 1	60	91.6%	NYHA, *BUN*, BB, ICD shocks
Ng 2012	Multicenter	RC	n.r.	424	69	88	27	100	131	66	62	100	2	84	92.5%	DM, NYHA, GFR, *peri-infarct zone*
Pacifico 1999	Single center US	RC	nr	421	63 ± 11	84	34 ± 12	82	nr	20	nr	0	2.1 ± 1.4	55	96.8%	Age, LVEF, ICD shocks
Panotopoulos 1997	Single center Wisconsin - US	RC	1983 - 1995	769	62 ± 10	80	33	79	nr	nr	11	30	3.8 ± 2.5	185*	93.5%	Age, LVEF, NYHA, ICD shocks

Supplemental Table S1. Continued.

Study	Population												Outcomes			Predictors *
	Source	**Study design**	**Time frame**	**N**	**Age Mean ± SD**	**% male**	**LVEF Mean ± SD**	**% ischemic CMP**	**QRS Mean ± SD**	**% β-blocker**	**% CRT**	**% primary indication**	**Follow-up (years) Mean± SD**	**n**	**1-year survival**	
Pellegrini 2008	Single center California - US	RC	1993 - 2003	502	62 ± 14	75	nr	44	nr	nr	11	30	4 ± 2.7	119	92.5%	Age
Pires 2006	Single center US	PC	1996 - 2004	835	65 ± 13	77	24 ± 11	57	nr	48	16	56	3 ± 2	182	91%	Age, sex, HF etiology, CABG, LVEF, BB, CRT, amiodarone, ICD indication, ICD shocks, *ICD testing*
Poole 2008	SCD-HeFT	RCT	1997- n.r.	811	60	75	24	52	n.r.	n.r.	n.r.	100	3.8	173	94%	ICD shocks, *Duke score, ECG intervals, drug abuse*
Robin 2006	Single center US	RC	1993 - 2003	556	63 ± 15	81	33 ± 15	54	nr	31	nr	47	2.2 ± 2.4	115*	92%	Age, sex, CRD, DM, LVEF, ICD indication
Rooselvelt Gilliam 2007	FH-Hrvar database US	PC	n.r	842	68 ± 11	88	n.r.	n.r.	n.r.	n.r.	100	n.r.	1	66	90.6%	*HR variability*

Supplemental Table S1. Continued.

Study	Population												Outcomes			Predictors *
	Source	**Study design**	**Time frame**	**N**	**Age Mean ± SD**	**% male**	**LVEF Mean ± SD**	**% ischemic CMP**	**QRS Mean ± SD**	**% β-blocker**	**% CRT**	**% primary indication**	**Follow-up (years) Mean± SD**	**n**	**1-year survival**	
Saxon 2010	ALTITUDE study	PC	2004-2009	185778	67 ± 13	74	n.r.	n.r.	n.r.	n.r.	42	n.r.	2.1 ± 1.6	17972	92%	ICD shocks, *remote follow-up*
Schefer 2008	Single center Switzerland	RC	1995 - 2006	176	53 ± 13	78	nr	0	nr	67	0	23	4.3 ± 3	32	90%	CRD, NYHA, LVEF, ACEI, amiodarone, ICD shocks
Solomon 2010	MADIT-CRT trial US	RCT	2004-2007	1372	64 ± 11	75	29 ± 3	55	158 ± 19	93	55	93	1.3	55	97.6%	*Changes in LVEF and LV dimensions*
Stein 2008	SERF registry US	PC	2001-2004	1655	69 ± 12	82	32 ± 12	n.r.	n.r.	68	n.r.	48	1	183	84%	Age, DM, NYHA, A Fib, LVEF, *BP, HR,* digoxin, diuretics, statins, *physical inactivity*
Sweeney 2010	PAINfree PAINfreeRx EMPERIC PREPARE	RCT	n.r.	2135	66 ± 12	80	32 ± 12	87	n.r.	68	n.r.	67	3.4 ± 2.8	141	93.3%	Age, NYHA, HF etiology, BB, ICD shocks

Supplemental Table S1. Continued.

Study	Population												Outcomes			Predictors *
	Source	Study design	Time frame	N	Age Mean ± SD	% male	LVEF Mean ± SD	% ischemic CMP	QRS Mean ± SD	% β-blocker	% CRT	% primary indication	Follow-up (years) Mean± SD	n	1-year survival	
Theuns 2011	2 centers Netherlands and Switzerland	RC-P	1999-2008	463	62 ± 11	75	24 ± 7	50	165 ± 30	76	100	75	2.5	85	93.7%	*Co-morbidity score*
Turakhia 2007	Single center California - US	RC	1993 - 2003	507	62 ± 14	75	nr	56	nr	nr	11	30	4 ± 2.7	120	93.2%	CRD, Creatinine, GFR
Tzeis 2011	LICAD study Germany	CC PC	1998-2003	236	59 ± 14	68	n.r.	63	n.r.	63	n.r.	17	6.1 ± 2.5	74	97%	Age, Sex, CRD, LVEF, NYHA, BB, ICD shocks, *depression*
Van Rees 2011	Single center Netherlands	RC	1996-2008	1544	61 ± 13	79	35 ± 16	63	125 ± 5	51	35	56	3.4 ± 21.5	423	n.r.	Age, CDR, A Fib, NYHA, LVEF, QRS, ICD shocks
Van Welsene 2011	Single center Netherlands	RC	1996-2008	2134	63 ± 12	80	32 ± 13	64	126 ± 33	55	40	61	3.4 ± 2.8	423	93.8%	ICD indication
Verma 2010	Single center ON - Canada	RC	2000-2007	421	68 ± 11	82	27 ± 9	75	n.r.	89	28	100	2.1 ± 1.3	46	n.r.	ICD shocks

Supplemental Table S1. Continued

Study	Population												Outcomes			Predictors *
	Source	**Study design**	**Time frame**	**N**	**Age Mean ± SD**	**% male**	**LVEF Mean ± SD**	**% ischemic CMP**	**QRS Mean ± SD**	**% β-blocker**	**% CRT**	**% primary indication**	**Follow-up (years) Mean± SD**	**n**	**1-year survival**	
Work 2007	Single center Denmark	RC	1996-2004	286	n.r.	84	n.r.	75	n.r.	64	0	88	1.9	58 †	94%	Sex, LVEF, A Fib, creatinine, digoxin, amiodarone
Zareba 2011	MADIT-CRT trial US	RCT	2004-2007	1817	64 ± 11	75	24 ± 5	55	158 ± 19	93	60	93	2.4	127	97.6%	CRT

* Only predictors whence the hazard ration was reported are cited. Predictors in *italic* means that these factors were not meta-analyzed due to single study report or use of different definitions.

† Marijon et al's studies analyzed HF deaths. Work et al reported on cardiac deaths.

LVEF, left ventricular ejection fraction; CMP, cardiomyopathy; CRT, cardiac resynchronization therapy; RC, retrospective cohort; RC-P, retrospective cohort on prospectively collected data; RCT, randomized controlled trial; CRD, chronic renal dysfunction, COPD, chronic obstructive pulmonary disease; HTN hypertension, DM, diabetes mellitus, PVD, peripheral vascular disease; HF, heart failure; ICD, internal cardiac defibrillator; NYHA, New York Heart Association; A Fib, atrial fibrillation; 6'WT, 6 minute walk test; RV, right ventricular; CABG, coronary artery bypass grafting; BP, blood pressure, HR, heart rate; ACEI, angiotensin converter enzyme inhibitors; BB, β-blockers; PH, pulmonary hypertension; GFR, glomerular filtration rate; BUN, blood urea nitrogen; ECG, electro-cardiogram; n.r., not reported.

Supplemental Table S2. Risk of bias of included studies

Study	Predictor selection	Candidate predictor selection	Over-fitting	Model assumptions	Linearity	Missing data	Loss of follow-up	Validation
Agarwal 2007	clinical	nr	yes	checked	nr	nr	0%	nr
Al-Khatib 2008	clinical	nr	no	nr	nr	7%, excluded	nr	nr
Arshad 2011	nr	nr	no	nr	nr	nr	nr	nr
Barsheshet HR 2011	clinical	full model	yes	nr	nr	21%, excluded	nr	nr
Barsheshet JCE 2011	clinical	full model	no	nr	nr	nr	nr	nr
Bhavnani 2010	clinical	full model	no	nr	nr	nr	13%	nr
Bilchick 2010	nr	nr	no	nr	nr	nr	0%	nr
Bilchick 2012	clinical	full model	no	nr	nr	nr	nr	nr
Blatt 2008	clinical	nr	yes	nr	nr	13%, imputed	nr	nr
Blendea 2009	nr	nr	yes	nr	nr	nr	0%	nr
Boriani 2012	statistical	nr	no	nr	nr	nr	nr	nr
Bocker 1998	nr	nr	yes	nr	nr	nr	nr	nr
Borleffs 2009	statistical	backward	no	nr	nr	nr	4.2%	nr
Borleffs 2010	nr	nr	no	nr	nr	nr	4.2%	nr
Brodine 2005	nr	nr	no	nr	nr	nr	0%	nr
Brullmann 2012	clinical	forward	no	checked	nr	nr	nr	nr

Supplemental Table S2. Continued.

Study	Predictor selection	Candidate predictor selection	Over-fitting	Model assumptions	Linearity	Missing data	Loss of follow-up	Validation
Bunch 2009	statistical	nr	no	nr	nr	nr	nr	nr
Chow 2008	clinical	nr	yes	nr	nr	nr	nr	nr
Coleman 2008	clinical	backward	no	nr	nr	nr	nr	nr
Cuculich 2007	statistical	forward	no	nr	nr	nr	nr	nr
Cygankiewicz 2009	statistical	nr	no	nr	nr	nr	nr	nr
Das 2010	statistical	full model	no	checked	nr	nr	nr	nr
Daubert 2008	statistical	nr	no	nr	nr	nr	nr	nr
Desai – AJC 2010	clinical	forward	yes	nr	nr	2%	nr	nr
Desai – JCPT 2010	clinical	forward	yes	nr	nr	nr	nr	nr
Dickinson 2007	clinical	backward	no	nr	nr	nr	nr	nr
Dubner 2005	nr	nr	no	nr	nr	nr	0%	nr
Duray 2009	statistical	forward	no	nr	nr	nr	nr	nr
Eckart 2006	nr	nr	no	nr	nr	nr	nr	nr
Exner 1999	nr	nr	no	nr	nr	nr	nr	nr
Exner 2001	clinical	nr	no	nr	nr	nr	nr	nr
Gatzoulis 2005	statistical	nr	no	nr	nr	nr	nr	nr
Goda 2011	clinical	full model	no	nr	nr	5%, excluded	0%	nr
Gold	nr	nr	no	nr	nr	nr	nr	nr
Hager 2010	nr	nr	no	nr	nr	0%	nr	nr
Ho 2005	statistical	nr	no	nr	nr	nr	nr	nr

Supplemental Table S2. Continued.

Study	Predictor selection	Candidate predictor selection	Over-fitting	Model assumptions	Linearity	Missing data	Loss of follow-up	Validation
Koller 2008	clinical	nr	no	checked	Blood pressure conformed to linearity	0%	0%	bootstrapping
Koplan 2006	statistical	full model	no	nr	nr	nr	nr	nr
Kramer 2012	statistical	forward	no	nr	nr	nr	nr	nr
Ladwiq 2008	clinical	full model	no	checked	nr	13%, excluded	nr	nr
Larsen 2011	statistical	forward	no	nr	nr	11%, excluded	nr	nr
Lee 2007	statistical	backward	no	checked	Age did not conform to linearity	nr	nr	nr
Lee 2012	statistical	forward	no	nr	nr	nr	nr	nr
Levine 1991	statistical	forward	no	nr	checked	nr	nr	nr
Levy 2008	nr	nr	no	nr	nr	5%, excluded	0%	nr
MacFadden 2012	clinical	full model	yes	checked	Age and LVEF conformed to linearity	13%, excluded	nr	nr
Marijon 2009	statistical	backward	no	nr	nr	nr	0%	nr
Marijon 2010	statistical	backward	no	nr	nr	nr	0%	nr
Mitchel 2003	statistical	nr	no	nr	nr	nr	nr	nr
Morrison 2012	statistical	forward	no	nr	nr	nr	nr	nr

Supplemental Table S2. Continued.

Study	Predictor selection	Candidate predictor selection	Over-fitting	Model assumptions	Linearity	Missing data	Loss of follow-up	Validation
Moss 2004	statistical	nr	no	nr	nr	nr	nr	nr
Ng 2012	statistical	backward	yes	nr	nr	nr	nr	nr
Pacifico 1999	clinical	backward	yes	nr	nr	nr	nr	nr
Panotopoulos 1997	nr	nr	no	nr	nr	nr	1.5%	nr
Pellegrini 2008	clinical	nr	no	checked	Checked, nr	~25%, treated as missing	nr	nr
Pires 2006	clinical	full model	no	nr	nr	nr	3%	nr
Poole 2008	clinical	full model	yes	nr	nr	nr	nr	nr
Robin 2006	clinical	full model	no	checked	nr	nr	nr	nr
Rooselvelt Gilliam 2007	nr	nr	no	nr	nr	30%, excluded	nr	nr
Saxon 2010	clinical	full model	no	nr	nr	8%, excluded	nr	nr
Schefer 2008	clinical	forward	yes	nr	nr	nr	0%	nr
Solomon 2010	clinical	nr	no	nr	nr	nr	nr	nr
Stein 2008	statistical	full model	yes	nr	nr	nr	nr	nr
Sweeney 2010	statistical	forward	no	nr	nr	nr	nr	nr
Theuns 2011	statistical	nr	no	checked	nr	nr	nr	nr
Turakhia 2007	clinical	nr	no	checked	nr	6%, excluded	nr	nr
Tzeis 2011	statistical	backward	yes	nr	nr	8%, excluded	nr	nr
Van Rees 2011	statistical	forward	no	nr	nr	nr	7%	nr

Supplemental Table S2. Continued.

Study	Predictor selection	Candidate predictor selection	Over-fitting	Model assumptions	Linearity	Missing data	Loss of follow-up	Validation
Van Walsene 2011	clinical	full model	no	nr	nr	nr	7%	nr
Verma 2010	statistical	forward	no	nr	nr	nr	nr	nr
Work 2007	statistical	nr	yes	nr	nr	nr	nr	nr
Zareba 2011	nr	nr	no	nr	nr	0.2%, excluded	nr	nr

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Supplemental Table S3: Summary of the effect of mortality predictors in ICD population

Predictors	**Including all the studies**				**Sensitivity analysis ***			
	HR	**95%CI**	**# of studies**	**I^2**	**HR**	**95%CI**	**# of studies**	**I^2**
Demographic characteristics								
Age								
per 10-year increase	1.49	1.35-1.65	20	80%	1.45	1.35-1.56	10	49%
Age >65 years vs. <65 years	1.61	1.32-1.96	4	39%	1.5	1.28-1.76	3	0%
Age >70 years vs. <70 years	2	1.67-2.4	4	0%	1.97	1.6-2.43	3	0%
Age >75 years vs. <75 years	2.7	1.48-4.92	3	90%	2.7	1.48-4.92	3	90%
Male Sex	1.12	0.99-1.28	13	53%	1.17	0.86-1.59	5	70%
Black race	1.23	1.08-1.41	3	34%	1.23	1.08-1.41	3	34%
Co-morbidities								
Renal dysfunction								
CRD (~<60ml/min)	2.08	1.69-2.55	9	78%	1.7	1.32-2.19	5	87%
CRD (ESRD)	2.87	1.90-4.33	6	73%	2.72	1.75-4.22	5	75%
GFR (per 15 ml/min)	1.48	1.27-1.74	7	82%	1.25	1.15-1.35	3	0%
Creatinine (per 1mg/dL)	1.63	1.03-2.6	3	93%	1.28	1.11-1.49	2	0%
Diabetes	1.63	1.46-1.82	16	56%	1.56	1.37-1.79	11	61%
COPD	1.54	1.38-1.71	4	36%	1.54	1.38-1.71	4	36%
Peripheral vascular disease	1.43	1.2-1.7	4	61%	1.43	1.2-1.7	4	61%
Hypertension	1.98	0.66-5.92	3	95%	1.26	0.46-3.43	2	94%
Heart failure characteristics								
NYHA								
II vs. I	1.36	1.05-1.76	5	61%	1.19	0.92-1.55	3	45%
III-IV vs. II-I	2.16	1.69-2.75	11	84%	1.87	1.25-2.78	3	91%
III vs. I	2.7	1.62-4.49	6	87%	1.93	1.17-3.18	3	75%
IV vs. I	6.08	3.27-11.3	2	36%	7.65	4.5-13	1	-

Supplemental Table S3. Continued.

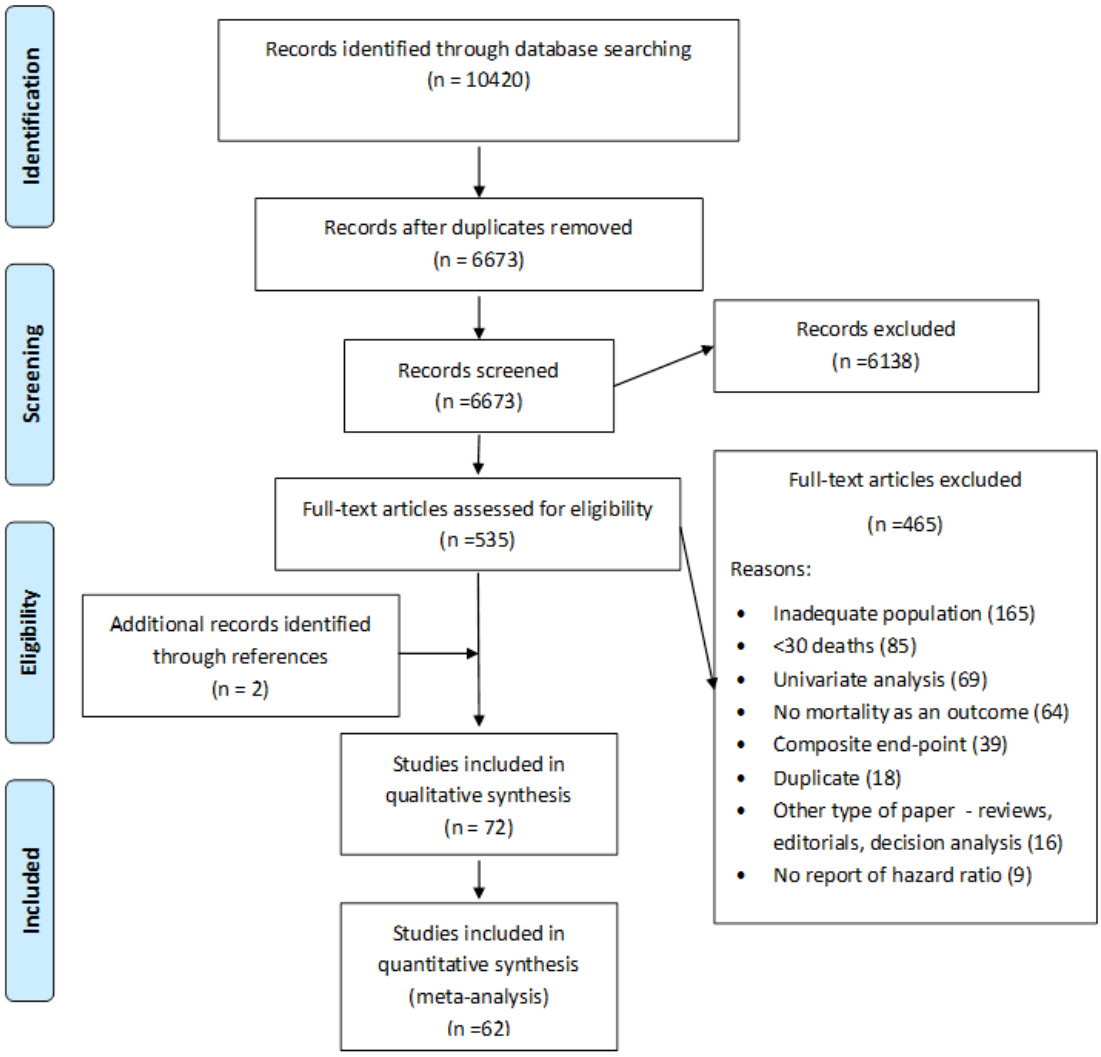
Predictors	Including all the studies				Sensitivity analysis *			
	HR	95%CI	# of studies	I^2	HR	95%CI	# of studies	I^2
LVEF								
per 10% increase	0.78	0.78-0.82	12	23%	0.77	0.73-0.83	10	38%
>20% vs. <20%	0.65	0.43-0.99	4	74%	0.51	0.27-0.97	3	80%
>30% vs. <30%	0.49	0.31-0.79	5	89%	0.37	0.21-0.66	1	-
>35% vs. <35%	0.41	0.29-0.58	3	0%	-	-	-	-
History of congestive HF	1.67	1.25-2.23	5	84%	1.67	1.25-2.23	5	84%
Ischemic cardiomyopathy	1.12	0.94-1.33	9	59%	1.21	0.96-1.54	4	76%
Atrial fibrillation	1.6	1.35-1.9	12	75%	1.47	1.25-1.72	7	70%
QRS > 120 msec	1.44	1.19-1.76	6	60%	1.33	1.1-1.61	4	51%
ICD characteristics								
Secondary vs. primary ICD	1.09	0.95-1.25	8	52%	1.11	0.93-1.32	5	69%
ICD therapy vs. no therapy								
ATP	0.83	0.52-1.31	3	48%	-	-	-	-
Inappropriate shocks	1.64	1.39-1.92	5	0%	1.55	1.29-1.86	3	3%
Appropriate shocks	2.17	1.68-2.81	9	86%	1.84	1.43-2.35	4	81%
Appropriate and inappropriate shocks	3.99	1.74-9.14	4	87%	2.34	1.59-3.44	1	0%
Appropriate or inappropriate	2.01	1.75-2.31	2	14%	2.09	1.83-2.39	1	0%
Electrical storm	2.26	1.57-3.25	3	0%	1.64	1.29-2.08	1	-

* Sensitivity analysis excluding studies with overfitting and studies including less than 4 strong predictors in their final models

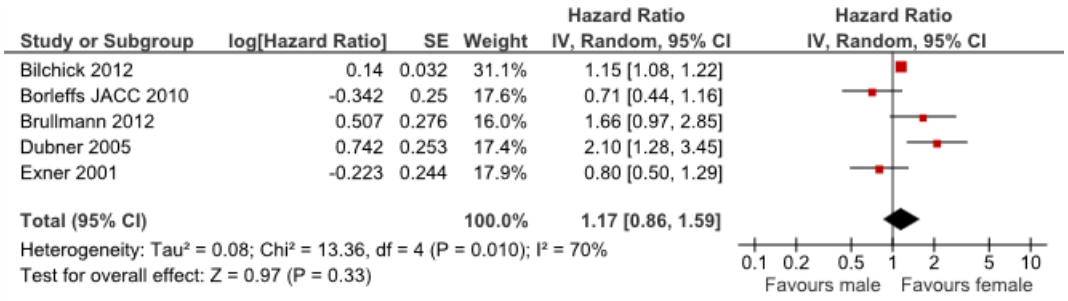
HR, hazard ratio; CI, confidence interval; CRD, chronic renal dysfunction; GFR, glomerular filtration rate; COPD, chronic obstructive pulmonary disease; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; HF heart failure; msec, milliseconds; ICD, internal cardiac defibrillator; ATP, anti-tachycardia pacing. .

SUPPLEMENTAL FIGURES:

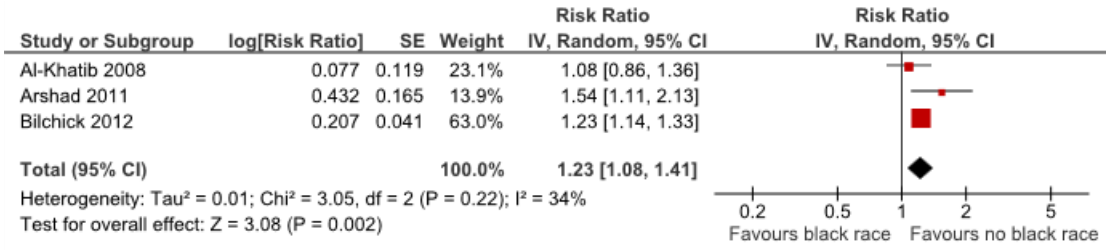
Supplemental Figure S1. Study selection flow



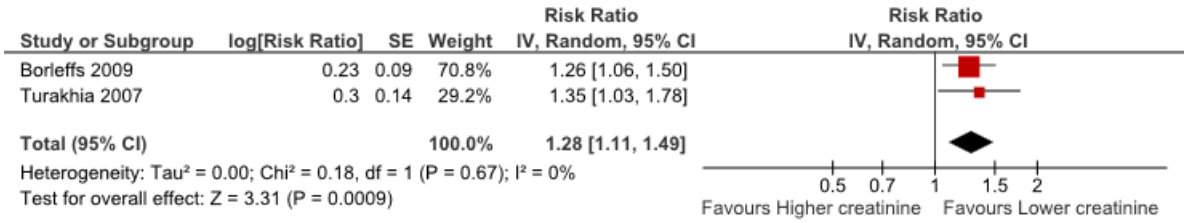
Supplemental Figure S2. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of male sex. Studies with overfitting and those with less than 4 strong predictors included in their models were excluded. A sensitivity analysis excluding the RCT cohort of Exner 2001 showed similar results.



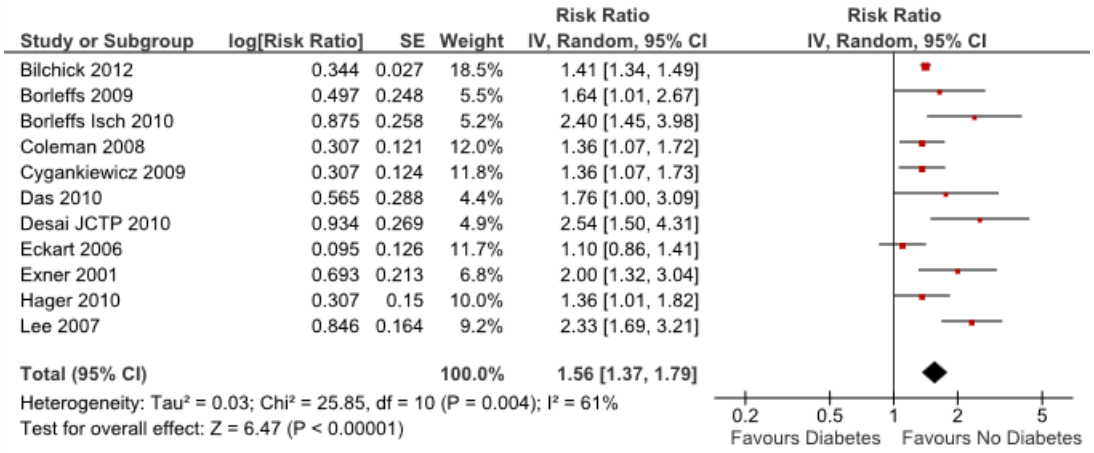
Supplemental Figure S3. Forest plot and pooled hazard ratio (HR) of the analysis of non-white race. There were not studies with overfitting or including less than 4 strong predictors their final models.



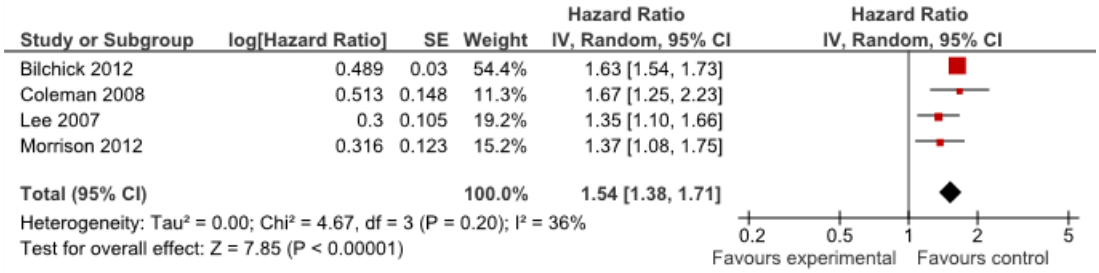
Supplemental Figure S4. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of creatinine. Studies with overfitting and those with less than 4 strong predictors included in their models were excluded.



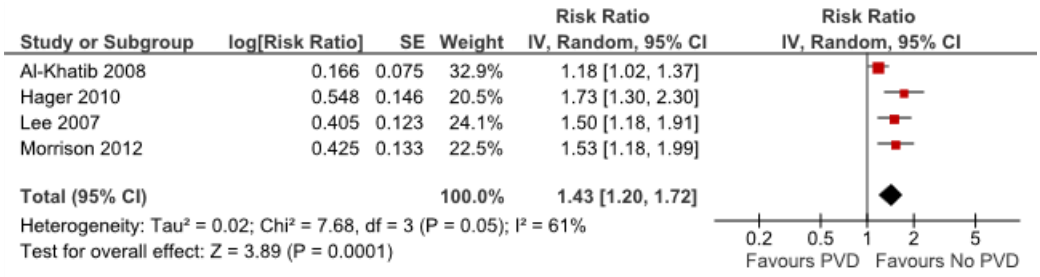
Supplemental Figure S5. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of diabetes. Studies with overfitting and those with less than 4 strong predictors included in their models were excluded. A sensitivity analysis excluding the RCT cohorts of Exner 2001 and Cygankiewicz 2009 showed similar results.



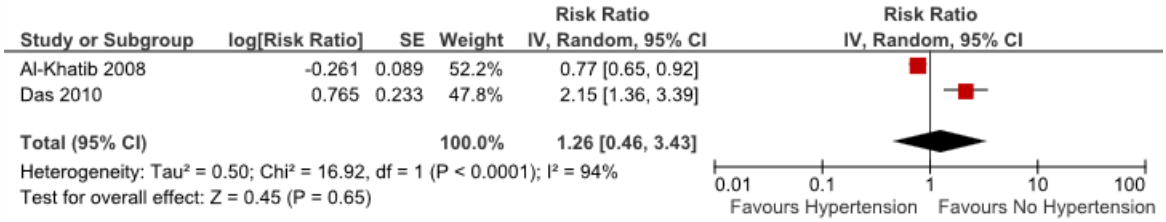
Supplemental Figure S6. Forest plot and pooled hazard ratio (HR) of the analysis of chronic obstructive pulmonary disease (COPD). There were not studies with overfitting or including less than 4 strong predictors their final models.



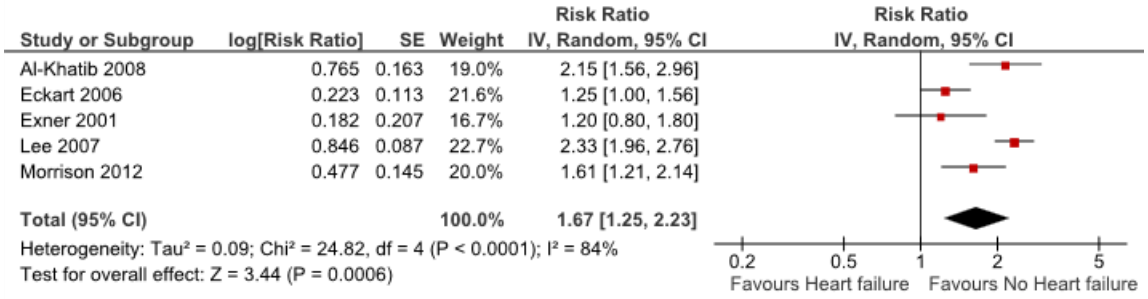
Supplemental Figure S7. Forest plot and pooled hazard ratio (HR) of the analysis of peripheral vascular disease (PVD). There were not studies with overfitting or including less than 4 strong predictors their final models.



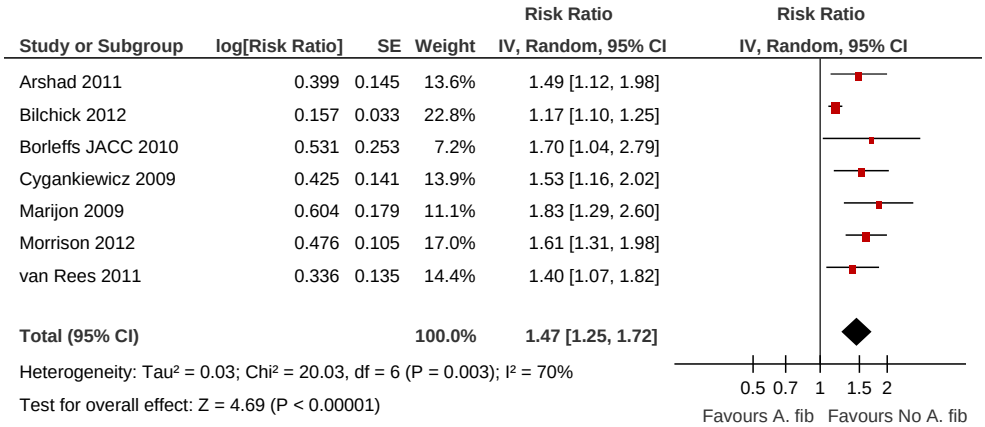
Supplemental Figure S8. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of hypertension. Studies with overfitting and those with less than 4 strong predictors included in their models were excluded.



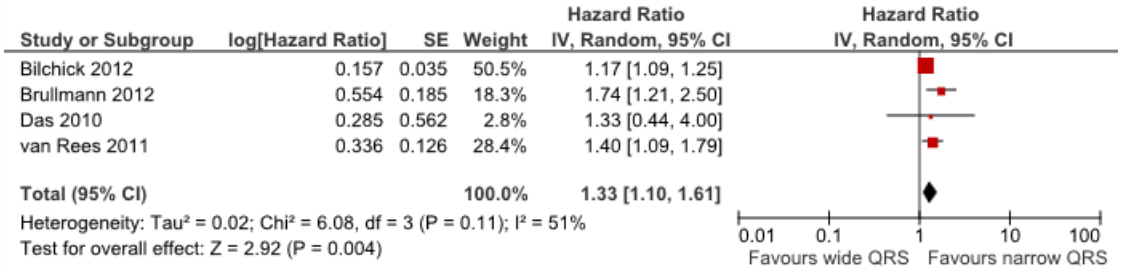
Supplemental Figure S9. Forest plot and pooled hazard ratio (HR) of the analysis of congestive heart failure (HF). There were not studies with overfitting or including less than 4 strong predictors their final models. A sensitivity analysis excluding the RCT cohort of Exner 2001 showed similar results.



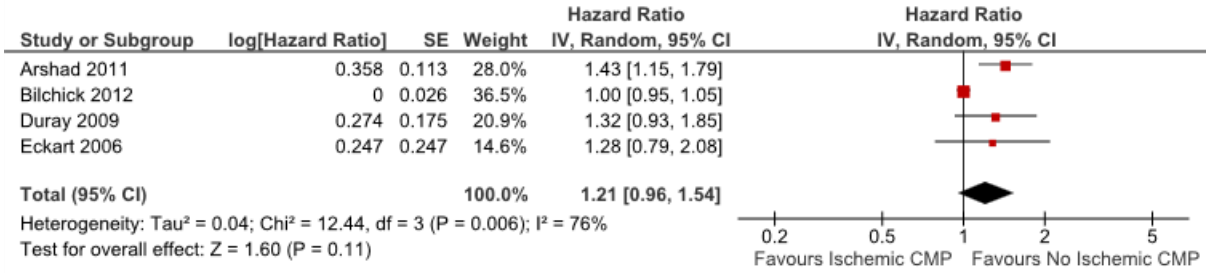
Supplemental Figure S10. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of atrial fibrillation (A Fib). Studies with overfitting and those with less than 4 strong predictors included in their models were excluded. A sensitivity analysis excluding the RCT cohorts of Arshad 2011 and Cigankiewicz 2009 showed similar results.



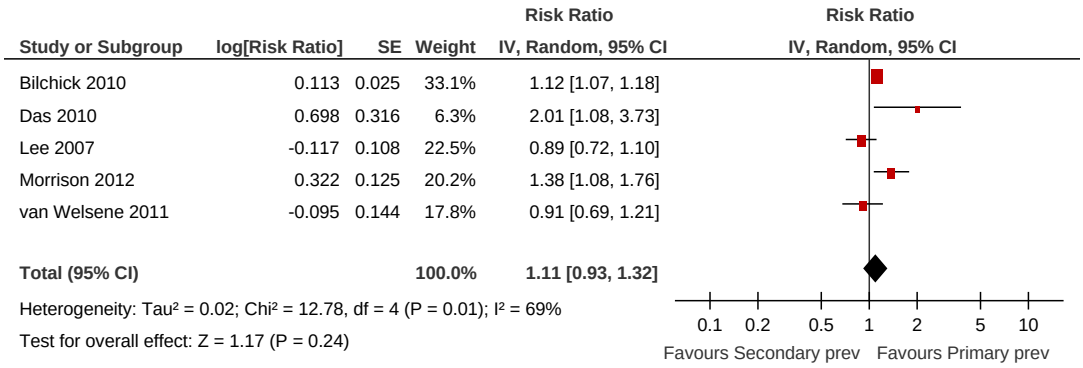
Supplemental Figure S11. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of QRS. Studies with overfitting and those with less than 4 strong predictors included in their models were excluded.



Supplemental Figure S12. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of ischemic cardiomyopathy (CMP). Studies with overfitting and those with less than 4 strong predictors included in their models were excluded. A sensitivity analysis excluding the RCT cohort of Arshad 2011 showed similar results.



Supplemental Figure S13. Forest plot and pooled hazard ratio (HR) of the sensitivity analysis of ICD indication (secondary vs. primary). Studies with overfitting and those with less than 4 strong predictors included in their models were excluded.



CHAPTER IV

The work described in this chapter has been submitted for publication to Circulation.

If accepted, the American Heart Association would own the copyright of this work.

Title: Predicting survival in heart failure patients with an implantable cardioverter defibrillator: The Heart Failure Meta-Score

Short title: The Heart Failure Meta-Score

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ABSTRACT

Background: Many heart failure (HF) patients are currently treated with an implantable cardioverter defibrillator (ICD) for preventing sudden death. Prognostic evaluation in HF is important to predict need for advanced therapies such as heart transplantation or mechanical circulatory support. . The aim of this study was to validate a prognostic score, derived from a meta-analysis, to predict survival in HF patients with an ICD.

Methods and Results: The HF Meta-Score includes 10 independent mortality predictors identified in a meta-analysis, including age, diabetes, chronic obstructive pulmonary disease, peripheral vascular disease, atrial fibrillation, NYHA, left ventricular ejection fraction (LVEF), renal function, QRS duration, and ICD shocks. The score was validated in 572 ambulatory ICD patients with reduced LVEF seen at a single institution from 2000-1011. The HF Meta-Score performance was evaluated in comparison to the SHOCKED predictors. During a median follow-up of 3 years, 139 patients died. The HF Meta-Score showed excellent calibration with predicted versus observed 1 and 3-year survival of 93% and 81.5% versus 92% and 81.5% respectively. Model discrimination was adequate (c-statistic of 0.704). The HF Meta-Score showed enhanced risk classification when compared to the SHOCKED predictors, with improved discrimination and calibration. The net reclassification improvement was 39%, 55% and 15% in patients categorized as having a 3-year predicted survival of 90-80%, 80-70% and <70%, respectively.

Conclusions: The HF Meta-Score provides an accurate assessment of survival in ICD HF patients. The excellent calibration and enhanced discriminatory capacity demonstrates the usefulness of the score for clinical decision making.

Key words: heart failure, survival, prognosis, defibrillation, score

INTRODUCTION

Heart failure (HF) is an increasing problem associated with significant morbidity and mortality. Moreover it is typically characterized by inexorable progression with a gradual decline in functional capacity until death [1]. When medical management fails, the main treatment options to improve survival are heart transplantation or mechanical circulatory support (MCS). The medical decision making process surrounding the need for advanced therapies throughout the course of HF is based on accurate prognostic assessment. However, even though transplantation or MCS are lifesaving, they are associated with risks and complications. Optimal prognostic assessment should therefore facilitate a favourable risk/benefit ratio.

Management of patients with HF is complex due to the increasing proportion of elderly patients with multiple co-morbidities, different patterns of disease progression, continuous improvement in medical management and development of new therapeutic options. These factors and their interactions result in challenges in the prediction of outcomes and consequently the decision-making process. To address these difficulties, investigators have developed a number of predictive models [2].

To our knowledge, all predictive models except two [3,4] were developed before the widespread use of implantable cardioverter defibrillators (ICD). This potentially compromises the ability of these models to accurately predict survival in current ICD treated HF patients. The use of ICDs has substantially increased in the past decade [5], with about 40% to 50% of HF patients currently being treated with an ICD [6,7].

The SHOCKED predictors, a score proposed by Bilchick et al [4], is a recently developed prognostic model derived from ICD patients included in the Medicare database. This model has demonstrated adequate performance. However, the exclusion of some important mortality predictors in ICD patients, such as QRS duration, the presence of peripheral vascular disease and ICD shocks during follow up may limit their applicability, performance and generalizability.

In order to enhance model generalizability and incorporate evidence based important predictors of mortality described in ICD patients, we constructed a predictive model from the results of a meta-analysis. In this report, we evaluate the value of our HF Meta-score to predict mortality in a cohort of ICD patients and validate its performance in comparison to the SHOCKED predictors.

METHODS

The Heart Failure Meta-score

We developed a predictive score by including the 10 mortality predictors identified in a meta-analysis on ICD patients [8]. This meta-analysis included 72 studies involving 257,692 ICD patients, and evaluated independent predictors of overall mortality. Briefly, the mean age of the population of the studies included in the meta-analysis varied from 65 to 72 years, between 72 and 87% were male, mean LVEF varied from 21 to 38%, between 55 and 72% had ischemic cardiomyopathy (CMP), 58 to 78% were using β-blockers and between 50 to 91% had a primary-prevention ICD. The pooled estimates of each predictor were obtained including only those studies that had used

multivariable analysis. These predictors included 3 continuous variables: age, left ventricular ejection fraction (LVEF) and glomerular filtration rate (GFR), and 7 dichotomous variables: diabetes, chronic obstructive pulmonary disease (COPD), peripheral vascular disease (PVD), atrial fibrillation, NYHA class III-IV, wide QRS (QRS >120 milliseconds) and ICD shock (appropriate or inappropriate shocks).

In order to calculate the HF Meta-Score, the β-coefficient (natural log of the pooled hazard ratio) of each predictor was added in a single score. Baseline survival (S_0, survival for score 0) was estimated by $S_0=e^{-\lambda t}$, where t=time and λ=the slope (survival change/year). The survival change per year (or slope) was estimated from the following sources: survival for a <75 years old patient, NYHA class III-IV, with an LVEF <20%, without chronic renal dysfunction (CRD), diabetes or COPD, and in sinus rhythm was derived from the baseline survival reported by Bilchick et al [4].; then survival for a 55 year-old patient, NYHA class II-I, with narrow QRS and absence of PVD and ICD shocks during follow-up was estimated from the associated risk and the expected distribution of age and NYHA class, prevalence of wide QRS [4] and PVD [9], and the incidence ICD shocks during follow up [10]. Based on this evidence the survival slope (λ) was set at 0.01157. A detailed description of the estimation of the survival slope is outline in the online Supplemental Material - Appendix A.

Survival at time t during follow-up for any score was then calculated by the following equation:

$$\text{Survival}_{(t)} = S_{0(t)}^{\,e^{(\text{HF Meta-Score})}}$$

To calculate the HF meta-score, each variable collected at the time of patient enrolment was multiplied by its β-coefficient and the products were summed. In patients without history of ICD shocks at baseline and receiving ICD shocks during follow up, the effect of ICD shock was entered as a time-dependent variable at the time of the first shock registered during follow up.

In the online Supplemental Material - Appendix B, we provide user-friendly tools, including an online calculator (www.hfmetascore.org), a Nomogram and a manual formula, to quantify individual patient risk.

Validation cohort and variables

The study population consisted of 572 consecutive ICD HF patients referred to our institution, a tertiary regional referral center for advanced HF patients, between 2000 and 2011. We excluded patients with a hospital admission due to decompensated HF in the 2 months prior to enrolment. We collected information related to clinical characteristics, laboratory values and hemodynamic variables by using electronic chart review of data closest to the enrolment date. The SHOCKED predictors score and survival was calculated by using the Nomogram provided by Bilchick et al [4]. We collected outcomes including death, ventricular assist device (VAD) implantation and heart transplantation.

Statistical analysis

Categorical variables were represented as proportions and continuous variables were summarised by their means and standard deviations (SD) or medians and inter-quartile ranges (IQR).

We assessed the HF Meta-Score model performance in comparison to the performance of the SHOCKED predictors by evaluating model discrimination and calibration. We fit a Cox proportional hazards model to predict mortality for each score. Follow up was censored at the time of VAD implantation, heart transplantation or last follow up visit to our institution. The primary attending physician was contacted to ascertain patients' status for those patients not seen at our institution for more than a year. The response rate was 88%. We then assessed the models' discrimination using Harrell's c-statistic. We evaluated the relative goodness-of-fit (GOF) of the two models by calculating the log likelihood, likelihood ratio (LR) and the AIC (Akaike information criterion). A higher LR and a lower AIC suggests better GOF. We used observed versus predicted survival to assess calibration.

We classified patients based on deciles of 3-year predicted survival (100-90%, 90-80%, 80-70% and <70%). We then used risk reclassification analysis (reclassification tables and reclassification calibration test) and net reclassification improvement (NRI) to assess global model performance of the HF Meta-Score in comparison to the SHOCKED predictors. Risk reclassification analysis is used to show how patients classified by the SHOCKED predictors were reclassified by the HF meta-score and compares the observed and predicted survival in each cross-classified category. This determines whether patients are reclassified correctly or due to chance. Observations are considered correctly classified if the observed rate is closer to the new (HF Meta-Score) than to the old (SHOCKED predictors) category. If the null hypothesis that predicted survival is equal to observed survival is rejected ($p \leq 0.05$), a model shows inadequate calibration [11]. The

NRI assesses if patients were reclassified in the correct direction, i.e. if survivors were reclassified as having better survival and deceased patients were reclassified as having lower survival. The NRI represents the difference in the proportion of patients correctly and inadequately classified [12]. We calculated an overall NRI and the NRI associated with each category. Because NRI is influenced by the categories used [13], we also estimated the continuous NRI based on a 1%-change in survival of the HF Meta-Score in comparison to the SHOCKED predictors. In this analysis, follow up was censored at 3 years.

The statistical analysis was performed using Stata IC 12 (Texas, USA) and R program 3.0.1. (New Zealand).

RESULTS

Validation cohort

Table 1 presents the baseline characteristics of the 572 ambulatory HF patients with an ICD. The mean age was 55 (SD 12 years, minimum 18 and maximum 82 years) and 441 (77%) patients were male. Sixty-four percent of patients were NYHA class III or IV; 46% patients had ischemic CMP. The mean LVEF was 23% (SD 6%) and 64 (11%) patients had atrial fibrillation. Overall, patients were on optimal medical therapy (90% were using β-blockers, 96% inhibitors of the renin-angiotensin system). Twenty-three percent of the patients had a history of inappropriate or appropriate ICD shocks at the beginning of follow up; and 147 (26%) patients received a first ICD shock during follow up. During a median follow up of 31 months (inter-quartile range of 47 months), 139

(24%) patients died, 82 patients underwent cardiac transplantation and 36 had a VAD implantation.

Predictive value of the Heart Failure Meta-Score

The HF Meta-Score ranged from values of 0 to 440 points with a median of 170 points (possible maximum of 520 points). The HF Meta-Score was significantly associated with mortality with a 9% increased risk for a 10-point increase in score (HR of 1.09 for a 10-point increase, 95%CI 1.07-1.11). The overall predicted survival was 93% and 81.5% at 1 and 3 years respectively, showing a nearly perfect calibration when compared to the observed survival (Figure 1). The observed survival was 92% and 81% at 1 and 3 years respectively. The HF Meta-Score model showed a discriminatory capacity assessed by the c-statistic of 0.704 (standard error 0.02).

The SHOCKED predictors score ranged from 0 to 260 points with a median of 77 points. The discriminatory capacity assessed by the c-statistic was 0.677 (standard error 0.02). The results presented in Table 2 suggest that discrimination (c-statistic) and GOF (assessed by the log likelihood, LR and the AIC) were better with the HF Meta-Score model than the model including the SHOCKED predictors.

Overall performance of the Heart Failure Meta-Score in comparison to the SHOCKED predictors

Table 3 shows the risk reclassification for patients stratified based on deciles of 3-year predicted survival by the SHOCKED predictors and the HF Meta-Score. The SHOCKED predictors did not place any patients in the highest survival category (predicted survival 100-90%). Of the 324 (57%) patients categorized with a predicted

survival of 90-80%, the HF Meta-Score reclassified patients by predicting better survival in 42% (135 of 324 patients) and reduced survival in 20% (51 of 324 patients). Of the 118 (21%) patients categorized in a predicted survival between 80-70%, the HF Meta-Score predicted better survival in 41% (49 of 118 patients) and reduced survival in 39% (43 of 118 patients). Of the 130 (23%) patients categorized in a predicted survival <70%, the HF Meta-Score predicted better survival in 65% (84 of 130 patients). The HF Meta-Score reclassified a total of 375 (66%) patients; the most frequent improved classification (80%) was observed in patients categorized by the SHOCKED as predicted survival of 80-70% (Table 4).

The assessment of the calibration of patients classified by the SHOCKED predictors showed inadequate calibration with significantly different observed and predicted survival (reclassification calibration test: χ^2= 22.5, p= 0.01) while the calibration of reclassified patients by the HF Meta-Score was better (reclassification calibration test: χ^2= 12.5, p= 0.25) suggesting that patients were correctly reclassified by the HF Meta-Score with a predicted and observed survival closer to the new risk category. Figure 2 shows how the HF Meta Score better stratified patients.

The NRI assesses the proportion of patients who were correctly classified, differentiating patients reclassified into correct or incorrect risk categories (Table 4). The HF Meta-Score correctly reclassified 238 (49%) patients and incorrectly classified 86 (18%) patients who survived more than 3 years, resulting in a relative improvement of 35%. Of the 89 patients who died during the 3 year follow up, the HF Meta-Score correctly reclassified 21 (24%) patients and incorrectly reclassified 30 (34%) patients,

yielding an overall change of -10%. Therefore, the overall NRI was 21% (31% – 10%) meaning that compared with the SHOCKED predictor categories, patients were 21% more likely to be better reclassified by the HF Meta-Score. The analysis of the NRI by category showed that the NRI was 39%, 55% and 15% in patients classified by the SHOCKED predictors as having a survival of 90-80%, 80-70%, and <70% respectively. The increased NRI in patients within the first 2 categories was due to better reclassification of both survivors and deceased patients; the NRI of 15% in patients classified as having a survival <70% by the SHOCKED predictors reflect misclassification of 21 (54%) deceased patients in that category. The analysis of the continuous NRI based on a 1%-change in survival was 36%, suggesting that patients were 36% more likely to be correctly reclassified by the HF Meta-Score, with survivors more likely to have higher predicted survival than deceased patients.

DISCUSSION

In this study, we describe the performance of a new tool, the HF Meta-Score, to predict survival in ICD treated HF patients. The HF Meta-Score includes 10 variables that were identified as strong independent predictors of mortality in a previous meta-analysis [8-no ref yet...but soon!]. The performance of this score showed adequate discrimination and excellent calibration. The HF Meta-Score showed superior capacity in accurately predicting survival in comparison to the SHOCKED predictors.

Calibration represents how similar predicted risk is to the observed risk. The HF Meta-Score showed excellent calibration as assessed by the risk classification test and the

survival curves comparing observed and predicted survival. The HF Meta-Score model's adequate calibration reflects its usefulness in determining the correct survival probability in a given patient. This should aid healthcare providers to direct resources to those patients with the greatest need. Adequate calibration allows patients and caregivers to assess the appropriate timing of diagnostic and therapeutic options.

Discrimination capacity reflects the ability of a model to differentiate patients who had the event from those who did not. The HF Meta-Score showed adequate discrimination based on the commonly accepted threshold value of the c-statistic, 0.70 [14]. The c-statistic has been criticized as being insensitive to improvements in model discrimination [11] and as having limitations in reflecting model performance in the clinical setting [12].

In this context, the risk reclassification tables and NRI may provide more useful information. The HF Meta-Score showed enhanced discrimination. The improved discriminatory capacity of the HF Meta-Score in comparison to the SHOCKED predictors reflects its inclusion of other important mortality predictors, including ICD shocks [10], QRS duration [4,15-17] and peripheral vascular disease [9]; and the use of age, renal function, LVEF as continuous rather than categorical predictors, which has been shown to provide more reliable estimation of the effect [8].

The HF Meta-Score also showed an overall 15% enhanced net discrimination in patients with a 3-year predicted survival <70%. In this risk category, the HF Meta-Score correctly reclassified 69% of patients surviving more than 3 years; however, there was a misclassification of 54% who died within 3 years. The implications of misclassification

may be the postponement of necessary therapies in high risk patients. The impact of model performance may be better explored using analytical techniques to guide medical decision making process [18]. Model discrimination may be improved by the addition of other predictors of mortality in high risk patients, including peak oxygen consumption [19,20] and brain natriuretic peptide (BNP) [21].

Other widely accepted prognostic models, which were initially developed in cohort of non-ICD patients, have been tested in ICD patients, and have shown varying degrees of discrimination. Goda et al [19] tested the performance of the Heart Failure Survival Score (HFSS) and reported a c-statistic of 0.69 (0.63-0.75), but model calibration was not evaluated. Other reports [21,22-24] have evaluated the performance of the Seattle HF Model (SHFM) and described that SHFM discrimination varied between 0.62-0.78. Assessment of SHFM calibration showed that the model may underestimate mortality in ICD patients [24]. Whether the HFSS or the SHFM perform better or worse than models derived specifically from ICD patients remains unexplored.

Study strengths and limitations

The HF Meta-Score was derived from the results of a meta-analysis. This should enhance generalizability and ensure more reliable model performance. However, we limited the variables included in the HF Meta-Score to those identified in the meta-analysis. Other important predictors, such as peak oxygen consumption and laboratory markers, that have limited available evidence about their mortality impact in ICD patients, may be important predictors and were not included in the HF Meta-Score. Future studies exploring these aspects are warranted.

In this report (Appendix B), we have provided different ways to calculate the HF Meta-Score. The use of only 10 variables offers the possibility of calculating the score by hand. The use of the nomogram and the web-based calculator greatly facilitates the estimation of survival at different time points.

CONCLUSIONS

In this report, we presented a new tool to accurately assess prognosis in ICD HF patients. The HF Meta-Score includes 10 easily available and important mortality predictors in ICD patients. The score showed excellent calibration and enhanced discriminatory capacity when compared to other ICD specific scores. The use of this model in the clinical setting may provide helpful information for medical decision-making.

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TABLES

Table 1: Baseline characteristics

Variable	**n= 572** mean ± SD / n (%)
Age (years)	55 ± 12
Male sex	441 (77)
Body mass index (kg/m^2)	28 ± 6
Cause of cardiomyopathy	
Ischemic	263 (46)
Idiopathic	224 (39)
Other *	85 (15)
Atrial fibrillation	65 (11)
NYHA class	
I	36 (6)
II	218 (38)
III	242 (42)
IV	76 (14)
Systolic blood pressure at rest (mmHg)	104 ± 16
Heart rate at rest (bpm)	70 ± 11
Left ventricular ejection fraction (%)	23 ± 7
Right ventricular systolic pressure (mmHg) †	44 ± 13
QRS duration (milliseconds)	143 ± 39
Wide QRS (>120 milliseconds)	379 (66)

Table 1. Continued.

Variable	**n= 572** mean ± SD / n (%)
Co-morbidities	
Diabetes	172 (30)
Chronic obstructive pulmonary disease	39 (7)
Chronic renal dysfunction	127 (22)
Peripheral vascular disease	66 (12)
Therapy	
β-blockers	513 (90)
ACE inhibitors or ARB	552 (96)
Digoxin	287 (50)
Spironolactone	308 (54)
Furosemide	476 (83)
Statins	304 (53)
Allopurinol	48 (8)
CRT	141 (25)
ICD shocks ‡	279 (49)
Laboratory values	
Hemoglobin (g/dl)	14 ± 1.5
Creatinine (mg/dl)	1.3 ± 0.4
Glomerular filtration rate (mL/min)	1.2 ± 0.5
Sodium (meq/L)	138 ± 4

Table 1. Continued.

Variable	**n= 572** mean ± SD / n (%)
Events during follow up	
Deaths	139 (24)
Heart transplantation	81 (14)
VAD	36 (6)

* Other causes of cardiomyopathy include chemo-induced, peri-partum, congenital, valvular and hypertensive cardiomyopathy.

†Information available in only 480 patients in whom right ventricular systolic pressure was technically feasible to measure.

‡ Of these 279 patients, 132 had a history of inappropriate or appropriate ICD shocks at the beginning of follow up and 147 received an ICD shock during follow up.

ACEI, angiotensin-converter enzyme; ARB, angiotensin II receptor blockers, CRT, cardiac

Table 2. Models' performance summary. Discrimination and goodness-of-fit.

Model	Goodness-of-fit			Discrimination
	Log likelihood	**Likelihood ratio chi^2 (p-value)**	**AIC**	**c-statistic**
HF Meta-Score	-745	66.5 (<0.001)	1491	0.704
SHOCKED predictors	-760	46.5 (<0.001)	1522	0.677

AIC, Akaike information criterion

Table 3. Risk reclassification table

		Heart Failure Meta-Score (HFMS)									
		100-90%		90-80%		80-70%		<70%		Total	
	SHOCKED	n	%	n	%	n	%	n	%	n	%
	100-90%	0	0%	0	0%	0	0%	0	0%	0	0%
Total	90-80%	135	42%	128	39%	46	14%	15	5%	324	57%
Deaths in 3 years		6		16		7		3		32	36%
Alive		129		112		39		12		292	60%
Predicted survival SHOCKED			85%		83%		83%		82%		
Predicted survival HFMS			93%		86%		77%		63%		
Observed survival at 3 years			**94%**		**84%**		**82%**		**66%**		
Total	80-70%	10	8%	39	33%	23	19%	46	39%	118	21%
Deaths in 3 years		0		3		4		11		18	20%
Alive		10		36		19		35		100	21%
Predicted survival SHOCKED			77%		77%		77%		77%		
Predicted survival HFMS			93%		85%		76%		62%		
Observed survival at 3 years			**99%**		**89%**		**77%**		**68%**		
Total	<70%	7	5%	39	30%	38	30%	46	35%	130	23%
Deaths in 3 years		1		10		10		18		39	44%
Alive		6		29		28		28		91	19%
Predicted survival SHOCKED			65%		62%		60%		54%		
Predicted survival HFMS			92%		84%		75%		51%		
Observed survival at 3 years			**83%**		**68%**		**68%**		**50%**		

The white diagonal cells indicate categories where the predicted survival by the SHOCKED predictors and the HF Meta-Score coincide. The green cells indicate categories where the predicted survival by the HF Meta-Score was higher than that predicted by the SHOCKED predictors. The red cells indicate categories where the predicted survival by the HF Meta-Score was lower than that predicted by the SHOCKED predictors. Observed survival was estimated from Kaplan-Meier life tables.

Table 4. Reclassification and net reclassification improvement (NRI)

		Total patients *		Patients reclassified by HF Meta-Score						NRI
				Higher survival		Lower survival		Net total of patients correctly reclassified		
SHOCKED		**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	
90-80%	**Total**	324	56.6%	135	42%	61	19%	196	60%	
	Deceased	32	36%	6	19%	10	31%	4	13%	
	Survivors	292	60%	129	44%	51	17%	78	27%	**39%**
80-70%	**Total**	118	20.6%	49	42%	46	39%	95	81%	
	Deceased	18	20%	3	17%	11	61%	8	44%	
	Survivors	100	21%	46	46%	35	35%	11	11%	**55%**
<70%	**Total**	130	22.7%	84	65%	0	0%	84	65%	
	Deceased	39	44%	21	54%	0	0%	-21	-54%	
	Survivors	91	19%	63	69%	0	0%	63	69%	**15%**
Total patients reclassified				268	47%	107	19%	375	66%	
Total deceased patients reclassified				30	34%	21	24%	-9	-10%	
Total survivors reclassified				238	49%	86	18%	152	31%	**21%**

The green cells indicate patients that were correctly reclassified (survivors as having better survival and deceased patients as having lower survival) by the HF Meta-Score. The red cells indicate patients that were incorrectly reclassified by the HF Meta-Score.

* The percentages are calculated based on a total number of patients of 572, a total number of survivors of 483 and a total number of deaths within the first 3 years of follow up of 89.

LEGENDS of FIGURES

Figure 1. Observed and HF Meta-Score predicted survival. The overlap between curves expresses virtually perfect calibration until 72 months. The divergence in the curves after 72 month follow up may be related to the small sample population (only 73 patients were followed for more than 72 months).

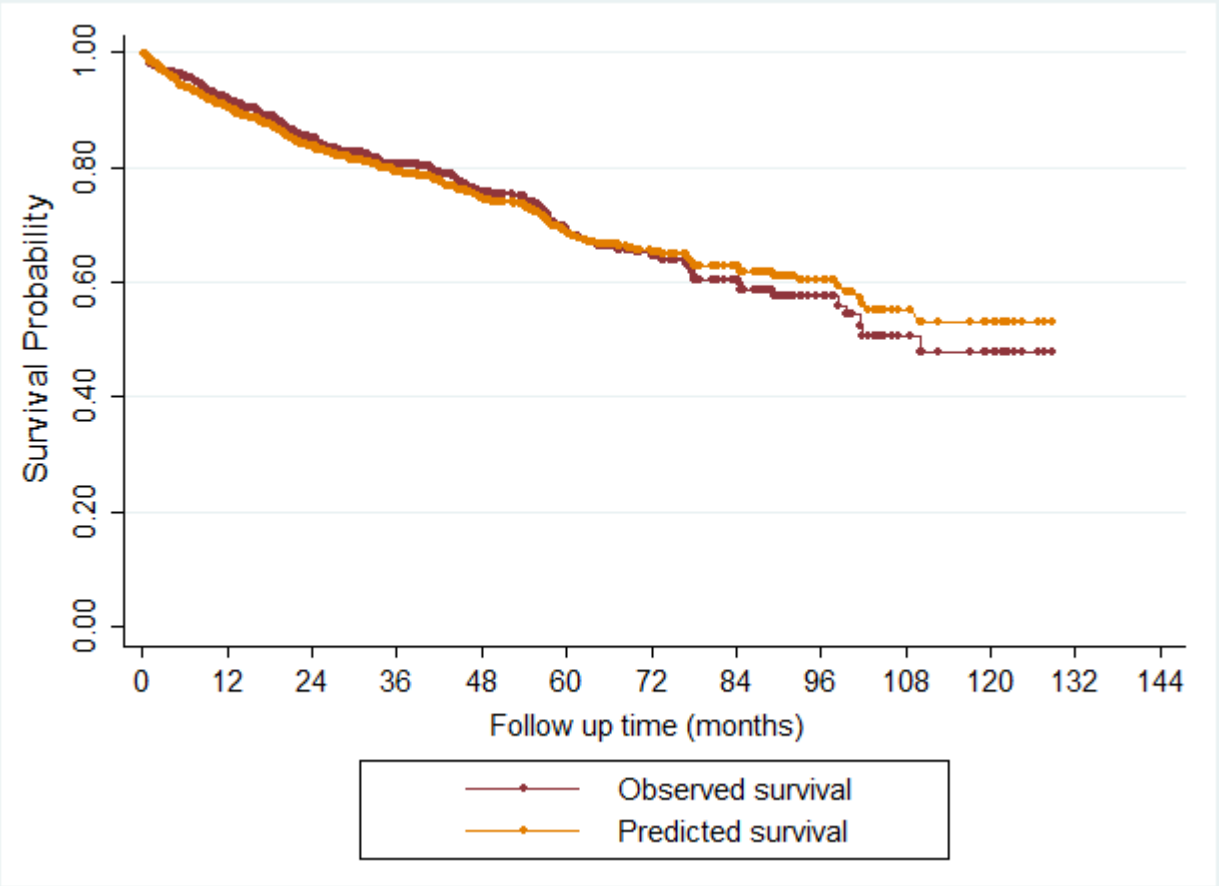
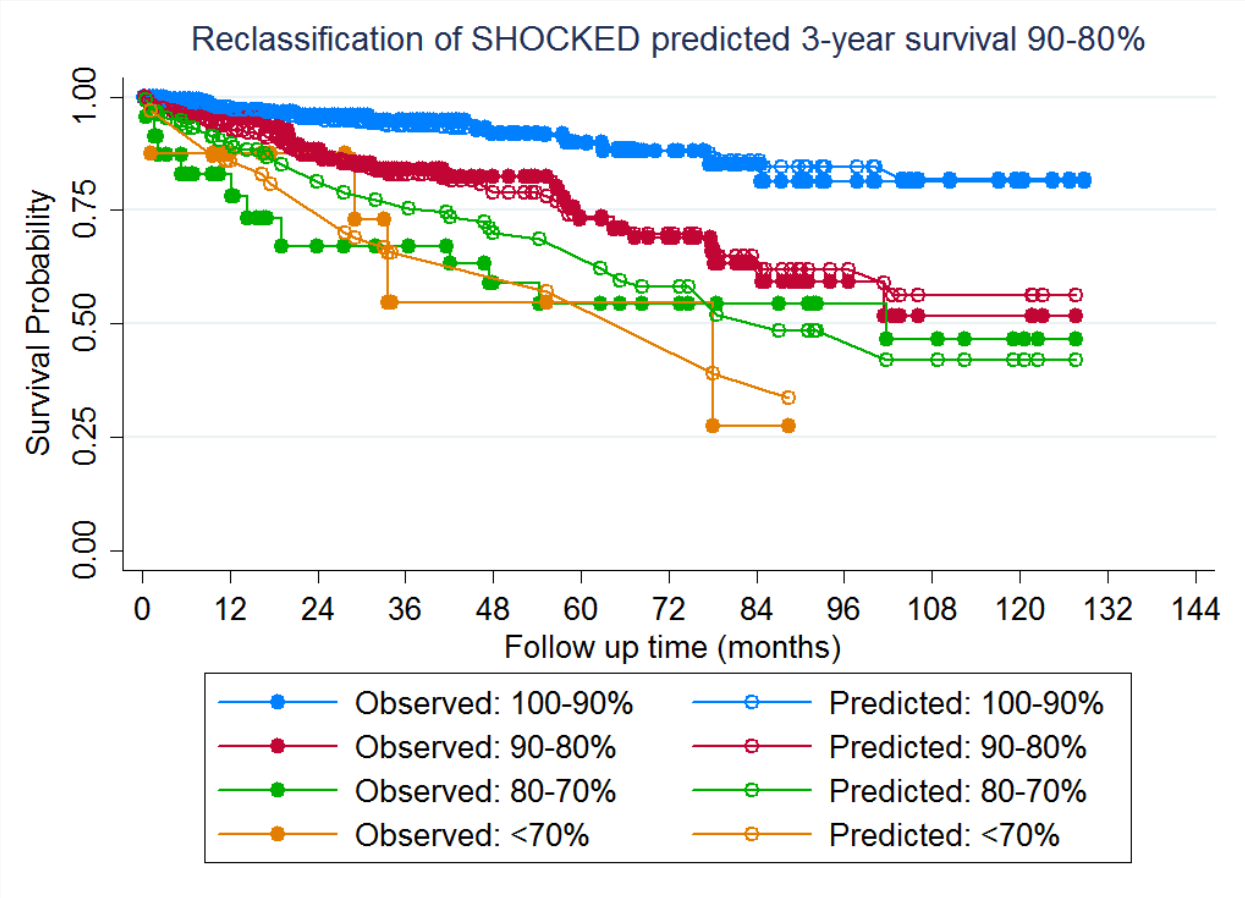
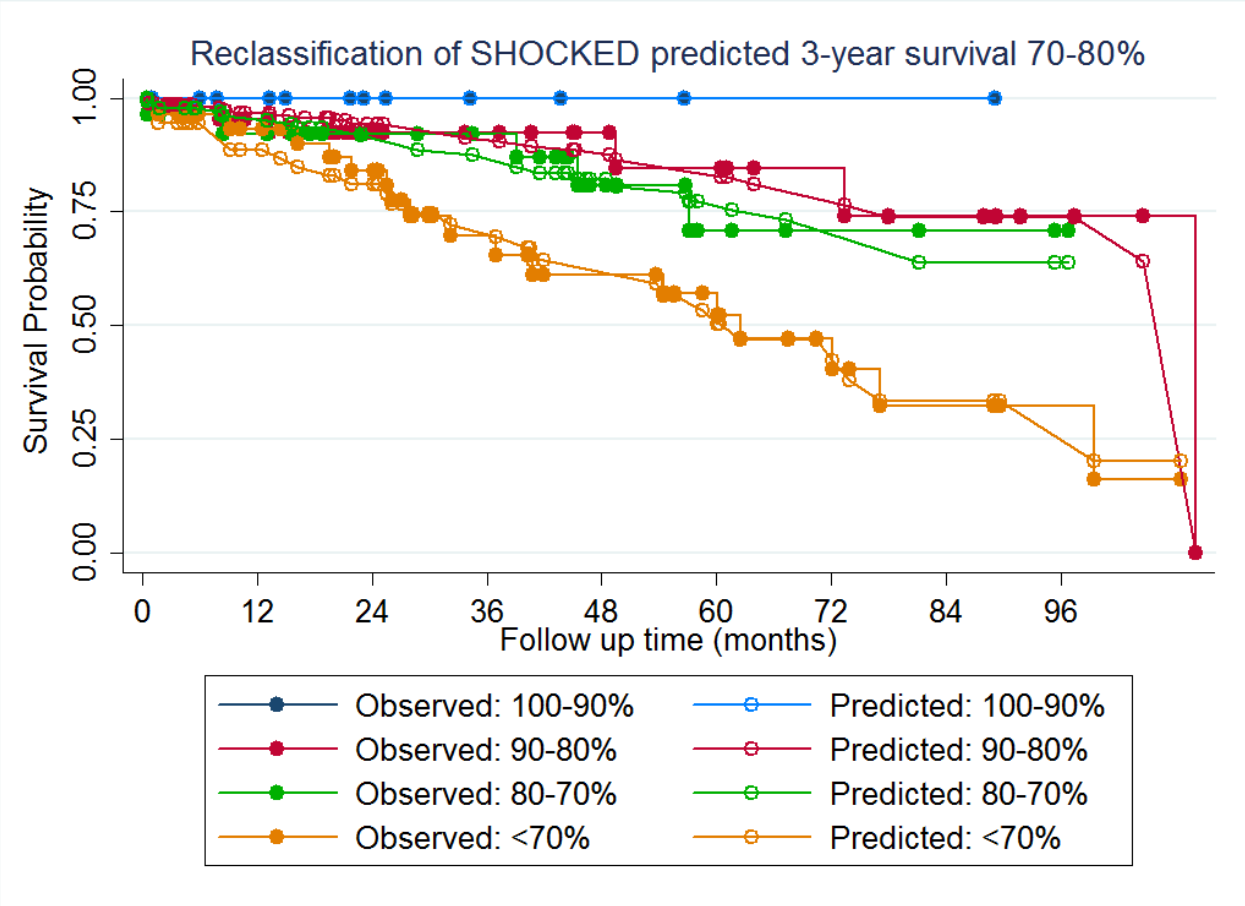


Figure 2. Risk reclassification calibration. The graphs show how patients were better reclassified by the Heart Failure Meta-Score by stratifying patients in different risk categories. In panel A, patients were classified as having a survival between 90-80% (red line) by the SHOCKED predictors. In panel B, patients were classified as having a survival between 80-70% (green line) by the SHOCKED predictors. In panel C, patients were classified as having a survival <70% (orange line) by the SHOCKED predictors.

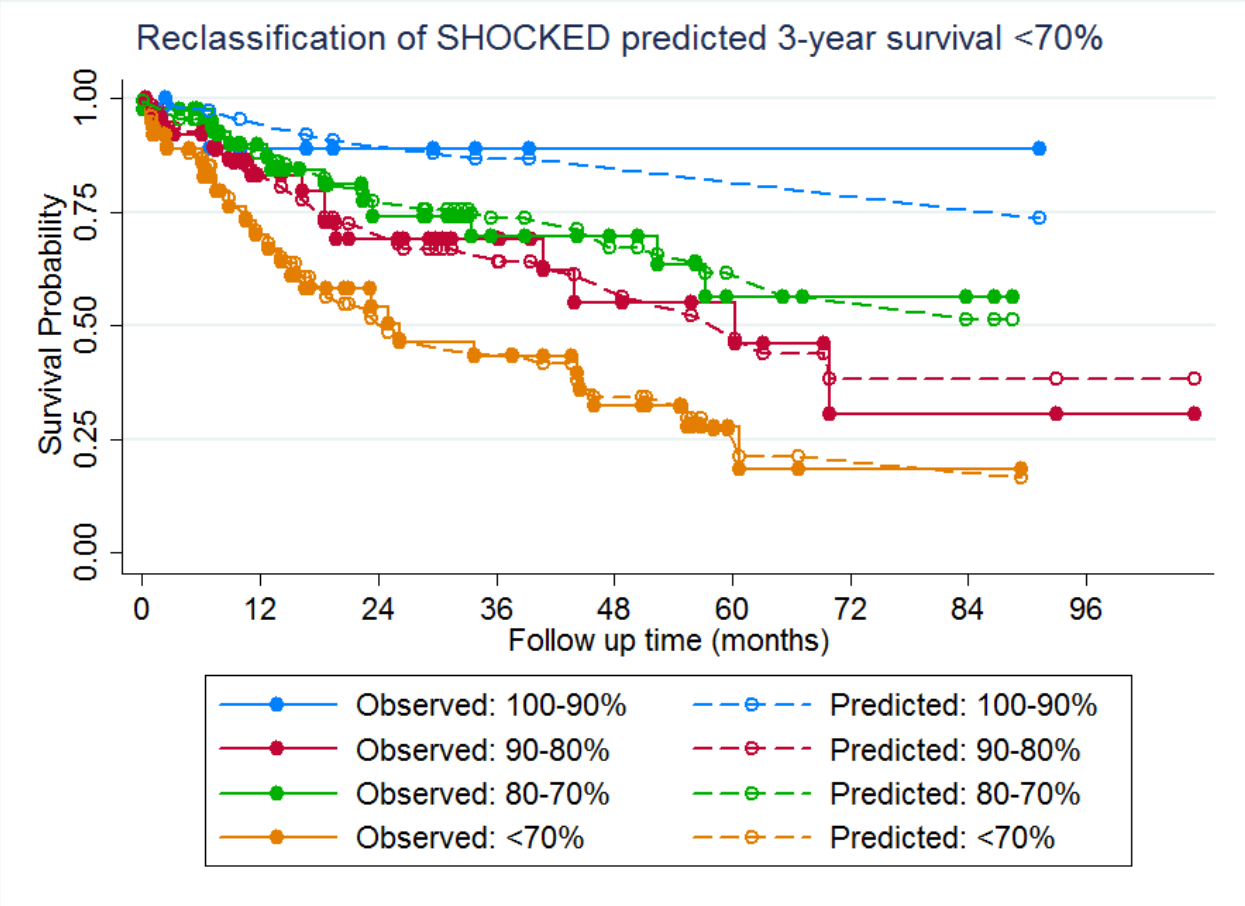
A



B


Reclassification of SHOCKED predicted 3-year survival 70-80%
Survival Probability
0.00
0.25
0.50
0.75
1.00
0
12
24
36
48
60
72
84
96
Follow up time (months)
Observed: 100-90%
Predicted: 100-90%
Observed: 90-80%
Predicted: 90-80%
Observed: 80-70%
Predicted: 80-70%
Observed: <70%
Predicted: <70%

C


Reclassification of SHOCKED predicted 3-year survival <70%
Survival Probability
0.00
0.25
0.50
0.75
1.00
0
12
24
36
48
60
72
84
96
Follow up time (months)
Observed: 100-90%
Predicted: 100-90%
Observed: 90-80%
Predicted: 90-80%
Observed: 80-70%
Predicted: 80-70%
Observed: <70%
Predicted: <70%

SUPPLEMENTAL MATERIAL

Appendix A

Estimation of the survival slope

The estimation of change in survival associated with a year (or slope λ) was obtained from the mortality risk reported by Bilchick et al [1]. The risk of patients without other specific characteristics (R_{wo}), was estimated based on the expected prevalence of specific characteristics using the following formula:

$$R_{wo} = \frac{R_t}{P_{wo} + HR \times P_w}$$

Where R_t represents the overall mortality risk at 1 year follow up, P_w and P_{wo} represent the proportion of patients with and without a specific characteristic respectively and HR represents the hazard ratio associated with the presence of a specific characteristic. Hazard ratios were obtained from a meta-analysis [2]. The expected prevalence of NYHA class II-I was 40.2% [1], of wide QRS was 41% [1] and of peripheral vascular disease (PVD) was 32% [3]; the expected incidence ICD shocks during follow up [4] was 14% at 1 year. Based on Bilchick et al [1], survival for a <75 year-old patient, NYHA class III-IV, with LVEF >20%, without chronic renal dysfunction (CRD), diabetes or chronic obstructive pulmonary disease (COPD), and in sinus rhythm was considered 0.955.

Table. Information used to estimate of change in survival associated with a year (or slope λ).

Patient characteristics	**Survival change / year (λ)**	**Hazard ratio (HR)**	**Proportion of patients with the bold characteristic (P_w)**	**Survival at 1 year**
<75 yo, NYHA class III-IV, LVEF >20%, no CRD, diabetes or COPD and in sinus rhythm	0.045	-	-	0.955
<75 yo, **NYHA class I-II**, LVEF >20%, no CRD, diabetes or COPD and in sinus rhythm	0.0296	1.87	40.2%	0.9704
<65 yo, NYHA class I-II, LVEF >20%, no CRD, diabetes or COPD and in sinus rhythm	0.02248	1.45	29.6%	0.9775
<55 yo, NYHA class I-II, LVEF >20%, no CRD, diabetes or COPD and in sinus rhythm	0.01739	1.45	35%	0.9826
<55 yo, NYHA class I-II, LVEF >20%, no CRD, diabetes or COPD, in sinus rhythm **and no ICD shocks during follow up**	0.01495	2.09	86%	0.985
<55 yo, NYHA class I-II, LVEF >20%, no CRD, diabetes or COPD, in sinus rhythm, no ICD shocks **and without PVD**	0.01314	1.33	68%	0.9868
55 yo, NYHA class I-II, LVEF >20%, no CRD, diabetes, COPD or PVD, in sinus rhythm, no ICD shocks **and with narrow QRS**	**0.01057**	1.43	59%	**0.98943**

yo, years old; NYHA, New York Heart Association; LVEF, left ventricular ejection fraction; CRD, chronic renal dysfunction; COPD, chronic obstructive pulmonary disease; ICD, internal cardioverter defibrillator; PVD, peripheral vascular disease.

Based on the information presented in the table and assuming that survival follows an exponential function, the survival change associated with a year (or the slope λ) was 0.01157.

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Appendix B

The Heart Failure Meta-Score:

Prediction of Survival in Heart Failure ICD patients

Example

Consider a 50 year-old diabetic male with NYHA class III heart failure and an LVEF of 40% who has received ICD therapy during follow up. Other characteristics include weight 80 kg, creatinine of 1 mg/dL and narrow QRS.

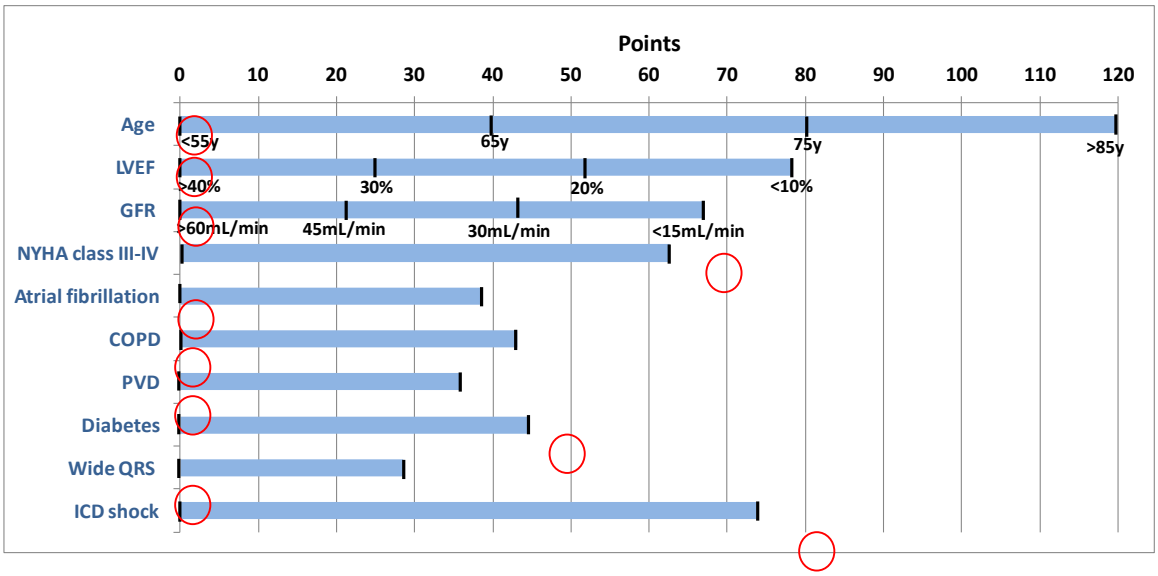
Online calculation of the HF Meta-Score

The HF Meta-Score is calculated by imputing patient's characteristics in each corresponding cell. An estimated survival curve for this particular patient is picture in the 1st Scenario. A 2nd Scenario is calculated assessing the potential impact of improved functional capacity (NYHA class I-II).

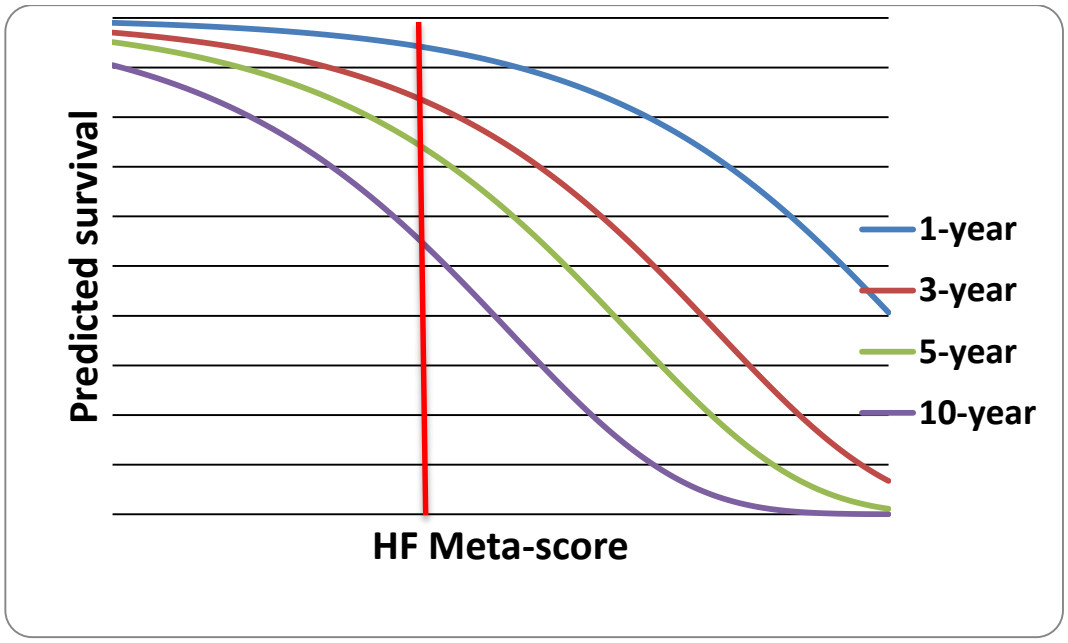
Calculation of the HF Meta-Score using a nomogram

The Seattle HF Score is calculated by summing the points associated with each factor.

Then that score is applied to the survival curves to obtain survival at different time points.



In this example, the adding points are 63 (NYHA) + 44 (Diabetes) + 74 (ICD shocks) = 181 points.



Manual calculation of the HF Meta-Score

The HF Meta-Score is calculated by multiplying the β-coefficient by the variable and summing the values. Binary predictors are coded as 1 if the characteristic is present and 0 if it is absent.

Predictor	β-coefficient	Applied example	Score
Age (per decade) *	0.40	0.40 * 0	0
LVEF (per 10%) ‡	0.26	0.26 * 0	0
GFR (pre 15 mL/min) †	0.23	0.23 * 0	0
COPD	0.43	0.43 * 0	0
PVD	0.36	0.36 * 0	0
Diabetes	0.44	0.44 * 1	0.44
Atrial fibrillation	0.39	0.39 * 0	0
NYHA class III-VI	0.63	0.63 * 1	0.63
Wide QRS	0.29	0.29 * 0	0
ICD shocks	0.74	0.74 * 1	0.74
		HF Meta-Score	**1.81**

* The β-coefficient represents the effect of age associated with 10-year change in patients older than 55 years. In patients younger than 55 years, the score associated with age is 0.

‡ The β-coefficient represents the effect of LVEF associated with 10-% change in patients with LVEF <40%. In patients with LVEF >40%, the score associated with LVEF is 0.

† The β-coefficient represents the effect of GFR associated with 15mL/min change in patients with GFR <60 mL/min. In patients with GRF >60 mL/min, the score associated with GFR is 0. GFR should be estimated using the MDRM formula.

After obtaining the score, you use the following formula to calculate survival at different time points. Here are illustrated, the calculus of survival at 1 and 3 years.

$$\text{Survival (1 yr)} = e(-\lambda * t)e^{(\text{HF Meta-Score})} = e(-0.01057 * 1)e^{(1.81)} = 94\%$$

$$\text{Survival (3 yr)} = e(-\lambda * t)e^{(\text{HF Meta-Score})} = e(-0.01057 * 3)e^{(1.81)} = 82\%$$

CHAPTER V

The work described in this chapter has been published in Expert Review in Cardiovascular Therapy. (Alba et al. Expert Rev Cardiovasc Ther 2012; 10:167-175.)

Title: Are endothelial progenitor cells a prognostic factor in patients with heart failure?

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ABTRACT

For the last two decades, endothelial progenitor cells (EPCs) have been proposed as a novel prognostic marker and potential therapeutic target in patients with cardiovascular diseases. EPCs are involved in the process of adult vasculogenesis and repair of dysfunctional endothelium. Endothelial dysfunction has been documented in the peripheral and coronary arteries of chronic heart failure (HF) patients and has proved to be an independent predictor of morbidity and mortality in HF patients. This has led researchers to analyze the association of EPCs and disease severity in HF patients. In this paper, we review studies analyzing the prognostic role of EPCs in patients with HF. Through a systematic search, we identified fourteen relevant studies. Only one study analyzed mortality as an outcome; the others evaluated the association between EPC levels and patients' characteristics. Overall, results were inconsistent and suggested that levels of EPCs may vary according to factors such as disease severity, underlying cause of cardiomyopathy and medical therapy.

Key words: Endothelial progenitor cells, Circulating progenitor cells, Heart failure, Prognosis

INTRODUCTION

In the 1990s, it was commonly accepted that postnatal angiogenesis occurred exclusively through the local outgrowth of pre-existing vessels by means of expansion of mature endothelial cells in response to angiogenic growth factors [1]. However, an enriched population of CD34+ cells, isolated from human peripheral blood, were subsequently shown to differentiate into endothelial cells in vitro and, in mice, were incorporated into areas of angiogenesis after ischemia [2]. In the double Id1/Id3 knock-out mice, bone marrow transplant reversed the failure to grow solid tumors due to poor vascular growth, thus demonstrating the involvement of bone marrow-derived cells in angiogenesis [3]. These findings provided the first direct evidence for the role of BM-derived and circulating cells in adult neovascularization and led to the possibility of novel therapeutic targets for tissue repair after ischemic injury.

With the discovery of circulating cells that contribute to the formation of new vessels, a radical change in the understanding of angiogenesis occurred. This new concept has led researchers to explore the role of this group of cells, known as "endothelial progenitor cells" (EPCs), in a variety of diseases in which endothelial function and angiogenesis constitute key aspects of pathogenesis. Endothelial dysfunction is implicated in the pathogenesis of heart failure and accumulating data have demonstrated the prognostic value associated with this abnormality. This paper summarizes current evidence from clinical studies analyzing the association and potential therapeutic role of EPCs in patients with heart failure.

Identification of endothelial progenitor cells

The term "endothelial progenitor cells" has commonly been used as a label for circulating blood cells identified by the expression of certain surface antigens as well as cultured mononuclear cells. Depending on the length of the time of culture, these cells can lead to at least two different cell populations: early-outgrowth EPCs and late-outgrowth EPCs.

Circulating endothelial progenitor cells:

Circulating EPCs are commonly characterized by the co-expression of the surface markers CD34, CD133 and VEGFR2 (vascular endothelial growth factor receptor 2). Although these markers are not unique to EPCs, it is generally accepted that their combination represents circulating EPCs [4].

Expression of CD34 was the first used to identify EPCs [2]. An important function of CD34 is cell-to-cell adhesion by binding of L-selectin. The expression of CD34 is variable and decreases as EPCs differentiate. The CD34 marker is not highly specific for EPCs as it is shared by other cells such as hematopoietic cells and mature endothelial cells [5,6].

CD133 is a five-transmembrane protein found on 20-60% of CD34+ cells. One of the identified functions of CD133 is as an organizer of membrane topology by regulating the lipid composition [7]. This marker is not expressed in mature endothelial cells. It can be also found on epithelial cells, hematopoietic and neuronal stem cells [8].

The VEGFR2, also known as KDR (kinase insert domain-containing receptor) in humans or Flk1 in rodents, is one of the three VEGFR family members. VEGFR2 is

present in cells involved in vasculogenesis that can differentiate to mature endothelial cells. This receptor binds VEGF, which participates in many functions of endothelial cells, including maturation and migration [9].

The cell surface phenotype CD34+CD133+VEGFR2+ is widely used to identify presumed circulating EPC in healthy and diseased subjects. However, the small quantity of circulating EPCs makes quantification difficult. These cells represent only 0.0001-0.01% of the peripheral blood mononuclear cells [4]. For this reason, many research studies report only CD34+VEGFR2+, CD34+CD133+ or just CD34+ cells as a measure of circulating EPCs. This heterogeneity in identifying and defining EPCs makes it difficult to compare results across studies.

The origin and function of EPCs are diverse and not completely understood. Lin et al [10] explored the origin of these cells by studying 4 patients who underwent a sex-mismatched bone marrow transplant (making possible the differentiation of circulating cells with donor or recipient genotype). They found that more than 95% of circulating EPCs had a recipient genotype while the expanded culture of late-outgrowth EPCs mostly displayed a donor genotype. Based on these results, they concluded that most of the circulating EPCs are vessel derived and a small proportion are BM-derived cells with high proliferative capacity. The different nature of these cells is further supported by a study showing that circulating EPCs (CD34+VEGFR2+ or CD34+VEGFR2+CD133+) did not correlate with cultured EPC levels in healthy individuals [11].

Several groups have examined the functional capacities of EPCs. CD34+CD133+VEGFR2+ cells do not have the capacity to form vessels in vitro nor in

vivo [12]; however, they do facilitate the process of angiogenesis probably through paracrine mechanisms [13]. This evidence supports the heterogeneous composition of this group of cells and creates controversy whether or not these cells should still be labelled as EPCs since they do not form vessels directly [14].

Early-growth endothelial progenitor cells:

EPCs have been cultured from cord blood, bone marrow and peripheral blood. Most researchers use density centrifugation (i.e. Ficoll centrifugation) to isolate peripheral blood mononuclear cells and then plate these cells. This assay identifies two different types of cells, early-outgrowth EPCs and late-outgrowth EPCs. Early-outgrowth EPCs are spindle-shaped cells obtained after 4-7 days of culture. Phenotypically, these cells have many characteristics of mature endothelial cells including but not limited to uptake of acetylated LDL and expression of CD34, VEGFR2, CD144, von Willebrand factor and CD31. However, these cells have limited proliferative capacity, do not form vessels directly, show phagocytic abilities and also express some hematopoietic markers such as CD45 and CD14. These features suggest that these cells represent a hematopoietic progenitor cell phenotype rather than a "true" endothelial progenitor cell phenotype [15].

Hill et al [16] modified the assay by adding a step of re-plating the non-adherent cells after 24-48 hours of culture in order to eliminate platelets and mature endothelial cells. Studies suggest that the cells obtained from both assays have similar characteristics [15,17].

Late-growth endothelial progenitor cells:

Late-outgrowth EPCs appear after 14 to 21 days of culture. They show cobblestone morphology, have a strong proliferative capacity, are capable of forming vascular networks and express endothelial like markers including VEGFR2, CD34, CD146 and VE-cadherin and do not express hematopoietic cell surface markers [17]. This phenotype suggests that late-outgrowth EPCs may constitute "true" EPCs. Interestingly, a study evaluating the potential of vasculogenesis using early- versus late-outgrowth EPCs in a mouse limb ischemic injury model demonstrated that the injection of late-outgrowth EPCs had significantly higher vessel forming capacity than early outgrowth EPCs. Moreover, this capacity was further increased when the two types of cells were implanted together [18]. This finding reinforces the potential paracrine function of early outgrowth cells in the process of vasculogenesis.

May EPCs play a pathogenic role in the development of heart failure?

The main role of EPCs is to promote vasculogenesis, repair endothelial loss and dysfunctional endothelium. EPCs increase after several stimuli including surgery [19,20], myocardial infarction [21-24] and burn injury [25]; migrate to areas of ischemic injury and participate in the process of vasculogenesis [4,26-28]. Furthermore, EPCs are also associated with endothelial function. Hill et al [16] demonstrated, in individuals with no clinical coronary artery disease (CAD), higher numbers of early outgrowth EPCs were associated with lower Framingham risk scores and better endothelial function measured by brachial reactivity. Endothelial dysfunction is widely accepted as an early

manifestation of atherosclerosis. Cheng et al [29] reported an independent association between low levels of early outgrowth EPCs and the presence of coronary and abdominal calcification in 889 healthy volunteers of the Framingham cohort.

Pathophysiologically, heart failure (HF) is characterized by reduced cardiac output and concomitant neuroendocrine activation. Endothelial dysfunction, defined as impaired vessel dilation to physiological stimuli, has been documented in the peripheral and coronary arteries of chronic HF patients [30-33], irrespective of the presence of CAD, and has been proposed as the cause of impaired vasodilatation in the coronary, pulmonary and peripheral vascular circulation [34]. Endothelial dysfunction is also an independent predictor of morbidity and mortality in HF patients [35,36]. It may be a critical part of the pathogenesis of HF resulting from increased oxidative stress, secondary to activation of the adrenergic/renin–angiotensin systems and to production of inflammatory cytokines [37].

Several therapies evaluated in non-randomized and randomized control trials, such as ACE inhibitors, β-blockers, statins, spironolactone, nitrates, and exercise, improve endothelial function in patients with HF. Developing a better understanding of the role of EPCs in HF, the mechanisms by which EPCs are capable of forming new vessels and repairing dysfunctional endothelium is critical in providing new insight into the complex pathophysiology of HF and potentially identifying new prognostic markers and therapeutic targets.

Studies evaluating the role of EPCs in patients with heart failure

Through a systematic search in Medline and references of selected studies, 14 studies that measured EPCs in patients with heart failure, utilizing any of the described assays, were identified. Twelve studies measured EPCs as circulating EPCs, using different combinations of the previously mentioned markers, and five studies measured early-outgrowth EPCs. There were no studies evaluating late-outgrowth EPCs in HF patients. One study analyzed mortality as an outcome. The remaining studies examined the relationship between levels of EPCs and patients' characteristics including but not limited to NYHA class, type of underlying cardiomyopathy and medical therapy. To optimize clarity, presentation and discussion of studies will be according to the assay used to measure EPC levels (circulating EPCs or early outgrowth EPCs). Table 1 summarizes the main characteristics related to population, study design, criteria used to define circulating EPC and results of studies evaluating circulating and cultured EPCs in heart failure patients.

Studies measuring circulating EPCs:

Valgimigli et al [38] were the first to publish an evaluation of the role of circulating EPC in HF patients. This cross-sectional study included 91 stable HF patients with impaired left ventricular ejection fraction (LVEF) and 46 sex- and age-matched healthy controls. They defined EPC according to the expression of CD34 and co-expression of CD34, CD133 and VEGFR2 antigens. They observed that NYHA class I or II patients had higher levels of circulating EPCs than healthy controls, while NYHA class III or IV patients had lower levels of EPCs. These results supported the a priori

hypothesis suggesting a "protective" role of EPC in the development of cardiovascular disease. The authors hypothesized that the presence of bone marrow exhaustion may be the underlying mechanism explaining lower levels of circulating EPCs in patient with NYHA class III and IV symptoms. Their finding that EPCs were inversely correlated with tumor necrosis factor- alfa (TNF-α) levels, a potent bone marrow inhibiting factor, supported this hypothesis [39]. These results were corroborated by Fritzenwanger et al [40] who conducted a similar study of 101 stable HF patients and 46 unmatched healthy controls.

Nonaka-Sarukama et al [41] analyzed circulating EPCs (CD34+ cells) in 22 acutely decompensated HF patients. In this study, NYHA class I and II patients had higher EPCs levels than controls and NYHA class III and IV patients had lower levels than controls. In the hospitalized NYHA class III-IV patients, EPC levels increased in response to HF treatment achieving values similar to controls.

Based on the results of these studies, the authors alleged that circulating EPCs were associated with disease severity in HF patients and that lower EPCs levels may be associated with a poorer prognosis. Results of an open-labelled randomized control trial conducted by Jie et al provided further support for the proposed causal mechanism of an exhausted BM [42]. They randomized 45 NYHA class II to IV HF patients with reduced LVEF (EF<50%) and cardiac renal syndrome (estimated creatinine clearance between 20 and 70 ml/min and mild anaemia) to receive Erythropoietin (15U/kg/week) for 1 year (n=30) or continue with standard medical therapy (n=15). Although Erythropoietin has been reported to increase EPC levels[43,44], the authors found that EPCs (CD34+ and

CD34+VEGFR2+) measured at 18 days and 1 year did not differ between treatment arms: in both groups, circulating EPCs showed a tendency to decrease over the year of follow up. This decrease was slightly but non-statistically significantly greater in the group of patients not receiving Erythropoietin. In addition, they found that baseline EP levels were lower than a group of healthy control individuals whom the authors studied. These observations support the hypothesis of an exhausted or suppressed BM accounting for the poor effect of Erythropoietin on EPC levels in advanced HF patients.

Based on this evidence, it was generally assumed that low levels of EPCs represent an adverse prognostic factor in heart failure. However, some studies have suggested that the nature of this association depends on the underlying cause of cardiomyopathy. Theiss et al [45] found that circulating EPCs were lower in patients with ischemic cardiomyopathy than idiopathic dilated cardiomyopathy in symptomatic HF patients with reduced LVEF but still higher than healthy controls. They did not find a correlation between NYHA class and EPC levels. In the same study, these investigators analyzed *in situ* concentration of homing factors including stromal cell derived factor-1 (SDF-1), hypoxia-inducible factor-1 (HIF-1) and vascular cell adhesion molecule (VCAM), in explanted hearts of transplant patients and observed that these factors were significantly upregulated (mRNA levels) in ischemic hearts but not in the myocardium from patients with dilated cardiomyopathy. This suggested an alternative hypothesis that circulating EPCs may be lower due to higher myocardial uptake and not just low generation or mobilization of EPCs in patients with ischemic cardiomyopathy. Other studies assessing only patients with CAD have reported that circulating EPCs are higher

in a group of patients with reduced LVEF of whom 55% of the patients were NYHA class III-IV [46,47]. This may contradict the hypothesis that circulating EPCs are a negative prognostic factor, at least in HF patients with CAD. Prospective studies evaluating outcomes may shed light on this ambiguity.

Other small studies have also contradicted previous results. Carvalho et al [48] measured CD34+ cells in 23 stable HF patients and did not find an association between EPCs and NYHA class. This may be related to the small sample size and low statistical power. Geft et al [49] analyzed CD34+ cells in 58 stable HF patients and failed to find an association between EPC and disease severity. They reported that patients with advanced NYHA class had a higher percentage of CD34+ apoptotic cells. These results suggest that increased oxidative stress present in patients with HF may induce cell damage without affecting EPC levels expressing a potential role of cell quality or functional capacity as a prognostic factor associated with disease severity. In this way, decreased EPC function may represent a factor associated with poorer prognosis. The origin of these apoptotic cells still remains unknown. They may be produced within the bone marrow which has been exposed to oxidative stress or constitute cells released by the dysfunctional endothelium.

Other studies have analyzed the impact of medical therapies on circulating EPC levels in HF patients. Tousoulis et al [50] performed a randomized control trial in symptomatic HF patients with LVEF <40% who were randomized to Rosuvastatin 10 mg/day, Allopurinol 300mg/day or placebo. They reported that circulating EPCs (CD34+VEGFR2+ and CD34+VEGFR2+CD133+ cells) were increased at 1-month in the

Rosuvastatin arm but not in the Allopurinol or placebo group in comparison to baseline values. They found that the increase in EPC levels was not associated with changes in the levels of inflammatory (fibrinogen, interleukin 6 and high-sensitivity C-reactive protein) and oxidative (myeloperoxidase and total lipid peroxides) markers.

Sarto et al [51] demonstrated that circulating EPCs (CD34+VEGFR2+CD31+) were increased after an 8-week anaerobic training schedule in 22 HF patients with LVEF <40%. They also described that the effect of exercise on EPC levels was not sustained since EPCs returned to baseline 8 weeks after discontinuation of the exercise activity. Craenenbroeck et al [52] found conflicting results: circulating EPCs measured as CD34+ or CD34+VEGFR2+ cells in 38 stable HF patients with LVEF <40% did not change after 6-months of anaerobic exercise training. These studies suggest that many factors may have an impact on circulating EPC levels. Discrepant results from small studies using unadjusted analysis make the interpretation of their results difficult.

In conclusion, some of the largest studies suggest that lower levels of circulating EPCs may act as a marker of disease severity. However, some studies suggest that this effect may be aetiology specific and differ in patients with ischemic cardiomyopathy. Other factors such as exercise activity and statins may modify EPC levels. In addition, the different cell phenotypes measured and the low number of CD34+VEGR2+CD133+ cells make difficult the comparison of results across studies. These results support the need for larger studies using adjusted analysis to better characterize the role of circulating EPCs in HF patients. There are no studies evaluating the association between circulating EPCs and HF outcomes, such as death, cardiac transplantation or HF hospital admission. This

gap in knowledge and the associated contradictory evidence make it difficult to make clear inferences.

Studies measuring early-outgrowth EPCs:

Few studies have evaluated the role of early-outgrowth EPCs. Table 1 cites the main characteristics of these studies. Valgimigli et al [38] demonstrated that lower levels of early-outgrowth EPCs were associated with decreased functional capacity measured by NYHA class in a group of stable HF patients with reduced LVEF. Similarly, an Israeli study by Shmilovich et al [53] reported a positive correlation between early-outgrowth EPCs and BNP levels in symptomatic HF outpatients. In this study, the authors also evaluated the effect of BNP on other functions of early-outgrowth EPCs from healthy individuals. They observed that cells from healthy individuals treated with BNP had increased cellular adhesion at low BNP concentration, increased migration, in vitro tubular formation and in vivo vascularisation in a mouse hindlimb ischemia model. These results suggested proangiogenic qualities of BNP and led to a hypothesis that in patients with advanced HF and higher levels of BNP, the peripheral uptake of EPC in areas of endothelial dysfunction and high vascular regeneration may explain the low EPC levels, similarly to Theiss et al's hypothesis [45] to explain low EPC levels in patients with ischemic CMP. However, there is very limited evidence to support this hypothesis.

In contrast to these results, the same Israeli research group [54] had previously conducted a cohort study in 107 stable symptomatic HF patients with systolic (n=79) and diastolic dysfunction (n=28) and found no association between type of LV dysfunction (systolic vs. diastolic), underlying aetiology of cardiomyopathy and levels of BNP. The

authors reported that higher levels of early-outgrowth EPCs were significantly associated with all-cause mortality and impaired NYHA class. However, the number of predictors (13) in their multivariable model on mortality was high compared to the small number of outcomes reported (21 deaths). This model overfitting may lead to find untrustworthy associations just by chance.

Similarly to some observations in circulating EPCs, levels of early-outgrowth EPCs may be modified by medical therapy. Sarto et al [51] reported that cultured EPCs were increased after 8-weeks of anaerobic exercise and gradually decreased 8 weeks after cessation of exercise. Jie et al [42] reported that administration of Erythropoietin tested in a randomized control trial was not associated with changes of early-outgrowth EPC levels during 1 year follow-up.

Two studies have evaluated the therapeutic function of intra-coronary EO-EPC injection in patients with myocardial infarction (MI) on LVEF. In the TOPCARE AMI trial [55], patients with an acute MI undergoing trans-cutaneous revascularization received an intra-coronary injection of EO-EPCs or bone marrow-progenitor cells. LVEF significantly improved by 9% to a greater extent than a matched reference group (change in LVEF of 2.5%) at 4 months' follow up. Conversely, in a pilot randomized placebo controlled trial, Assmuss et al [56] did not find an improvement in LVEF after intra-coronary EO-EPC injection in patients with a prior MI ≥ 3-month-old. They did find a significant improvement in LVEF with BM-progenitor cells administration. Differences in results between these trials may be related to the timing of EPC injection (during an acute MI or 3 months later) and study design (observational study versus randomized

controlled trial, respectively). Whether EPC therapy may have an effect on ventricular function in other types of cardiomyopathy remains unexplored.

Expert commentary

There is no doubt that circulating blood cells participate in vasculogenesis and vascular repair. However, there are still some areas of uncertainty. Inconsistent results are not restricted to HF patients; conflicting data also exist for example in patients with CAD. Chen at al [29] conducted one of the largest studies measuring circulating EPCs. This population based study of 889 subjects clinically free from CAD found no association between EPCs (CD34+ and CD34+VEGFR2+) and coronary and abdominal aorta calcification. However, low levels of circulating EPCs (CD34+VEGFR2+) were shown to be related to high cardiovascular risk in a cohort of 519 patients with different degrees of CAD [57]. Hristov et al [56] in a study of 144 stable CAD patients reported that initiation of statins decreased circulating EPC whereas a previous small study by Vasa et al [58] on a similar population reported a stimulating effect by statins on circulating EPCs. Similar inconsistencies occur in studies analyzing cultured EPCs. Xiao et al [59] reported that early-outgrowth EPCs were increased in patients with several cardiovascular risk factors; however other studies have reported an inverse association between early-outgrowth EPCs and arterial calcification [29] or cardiovascular events [57].

Prognosis assessment in patients with HF remains a challenge because of the dynamic nature of the process in addition to the existence of some unexplained variance

in outcomes. Hence, efforts are directed at the identification of new prognostic markers and their potential additive predictive value in order to refine this process. The concept of adult vasculogenesis and the interactive role of EPCs with vascular health suggest EPCs may be an attractive novel prognostic marker. However, the not yet fully understood role of EPCs and the ongoing inconsistent results create uncertainty about the potential use of EPCs as a prognostic factor. There is a need for additional evidence to establish or refute the role of EPCs as a prognostic marker and therapeutic target in patients with HF.

Five-year overview

EPCs represent an innovative marker with potential prognostic and therapeutic value. Even though there has been a substantial increase in the body of evidence in the last few years, further basic research studies are required to clarify the origin, function of EPCs and molecular pathways, to refine the EPC identification and characterization and to understand the process of adult vasculogenesis. Future studies should use of the most common assays to measure EPCs (EO-EPCs and circulating CD34+ cells co-expressing CD133 and /or VEGFR2 antigens) until unique markers to identify EPCs are determined. In addition, higher quality clinical studies using larger sample size to allow adjusted analysis, focusing on clinically important outcomes will help to clarify the potential future use of these cells. Although initial studies have shown benefit in the treatment of myocardial infarction with EPC injection, the role of EPCs in patients with HF is unclear.

Carefully designed studies analyzing their therapeutic qualities might be premature at this stage but certainly remain a possible future target based on the

acquisition of a more nuanced understanding of the role of EPCs. Increasing evidence suggests that cEPCs and EO-EPCs represent different cells. Future studies focused on understanding the biological and mechanistic role of these cells in adult angiogenesis under normal and abnormal circumstances will help to refine their clinical significance.

Key issues

- Endothelial progenitor cells (EPCs) are **peripheral blood circulating** cells involved in the process of adult vasculogenesis and repair of dysfunctional endothelium.
- Endothelial dysfunction plays a pathophysiologic role in heart failure (HF) and constitutes an independent predictor of morbidity and mortality.
- The nomenclature of EPCs still remains controversial. Better understanding of their biology is likely to help in clarifying the nomenclature.
- The role of EPCs, measured as circulating EPCs or early-outgrowth EPCs, as a prognostic marker in HF is not yet clear.
- Levels of EPCs may vary according to factors such as disease severity, underlying cause of cardiomyopathy and medical therapy.
- Few studies have evaluated the association between EPCs and important clinical outcomes such as death in patients with HF.
- EPCs offer potential in both improving prognostication and therapy. Exploration of the role of EPCs is still in its early stages.

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*** of interest**
**** of considerable interest**

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Table 1. Studies on heart failure patients measuring endothelial progenitor cells (EPCs)

Study	**Design**	**Population**		**EPCs**		**Outcome**	**Adjusted analysis**	**Results**
		n	**Characteristics**	**Circulating**	**Early-growth**			
Valgimigli 2004 [37]	Cross-sectional	136	91 stable HF patients with impaired LVEF and 45 sex- and age-matched controls. Statins discontinued for 3 weeks	CD34+ CD34+CD133+ VEGFR2+	Yes*	EPC levels according to NYHA class	No	Circulating and early-outgrowth EPCs increased with increasing NYHA class CD34+ correlated with BNP levels and peak VO_2 No association with medications or cytokines
Nonaka-Sarukama 2006 [40]	Cross-sectional	48	26 acutely decompensated HF patients and 22 unmatched healthy controls. Exclusion of patients with CAD	CD34+	No	EPC levels according to NYHA class	No	EPCs were increased in NYHA class I-II vs. III-IV EPCs increased after treatment in NYHA class III-IV BNP and erythropoietin levels were higher in NHYA class III-IV

Table 1. Continued.

Study	**Design**	**Population**		**EPCs**		**Outcome**	**Adjusted analysis**	**Results**
		n	**Characteristics**	**Circulating**	**Early-growth**			
Sarto 2007 [50]	Cohort	22	Stable HF patients, NYHA class II and III, LVEF<40% and peak VO_2<25ml/kg/min	CD34+VEGFR2+CD31+	Yes*	EPC levels before and after 8-week aerobic exercise training	No	Circulating and early-outgrowth EPCs increased after training and returned to baseline after exercise was stopped
Michowitz 2007 [53]	Cohort	107	Clinically diagnosed HF patients NYHA class II-IV	No	Yes*	Mortality	Yes	EPCs were associated with higher mortality and decreased NYHA class EPCs did not correlate with BNP levels

Table 1. Continued.

Study	Design	Population		EPCs		Outcome	Adjusted analysis	Results
		n	Characteristics	Circulating	Early-growth			
Theiss 2007 [44]	Cross-sectional	50	HF patients with LVEF<40% (15 patients with ischemic CMP and 25 patients with dilated CMP) and 10 healthy controls	CD34+CD133+ CD34+CD31+ CD34+CXCR4+	No	EPC levels according to underlying CMP	No	EPCs were higher in dilated CMP
Bulut 2008 [46]	Cross-sectional	35	Stable CAD patients with and without reduced LVEF (<or>50%)	CD34+VEGFR2+	No	EPC levels according to LVEF	No	EPCs were higher in patients with reduced LVEF Inverse correlation between EPCs and endothelial function in patients with both reduced or preserved LVEF

Table 1. Continued.

Study	Design	Population		EPCs		Outcome	Adjusted analysis	Results
		n	Characteristics	Circulating	Early-growth			
Geft [48] 2008	Cross-sectional	81	58 patients with clinically diagnosed HF and 23 age-matched healthy controls	CD34+ (percentage of apoptotic CD34+)	No	EPC levels and apoptotic cells according to NYHA class	No	No differences in early apoptotic EPC percentage Higher percentage of late apoptotic EPCs in NYHA class III-IV
Carvalho 2009 [47]	Cross-sectional	23	Clinically diagnosed HF patients	CD34+	No	EPC levels before and at peak exercise	No	EPCs were not correlated with NYHA class, peak VO_2 and LVEF. EPC levels did not change during exercise

Fritzenwanger 2009 [39]	Cross-sectional	142	101 patients with clinically diagnosed HF with impaired LVEF and 41 unmatched healthy controls	CD34+ CD133+ CD34+CD133+	No	EPC levels according to NYHA class	No	EPCs decreased with age CD34+ and CD34+CD133+ decreased with increasing NYHA class No significant differences according to the presence of CAD
Pellicia 2009 [45]	Cross-sectional	88	68 stable CAD patients and 20 healthy controls. Exclusion of patients with LVEF<25%	CD34+ CD133+	No	EPC levels according to LVEF (>or <45%)	No	EPCs were higher in patients with low LVEF
Shmilovich 2009 [52]	Cross-sectional	34	Clinically diagnosed HF patients NYHA class II to IV without statins treatment	No	Yes	EPC levels according to BNP levels	No	Positive correlation between EPCs and BNP levels

Craenenbroeck 2010 [51]	Cohort	48	38 HF patients with LVEF <40% and 10 age-matched healthy controls	CD34+ CD34+VEGFR2+	No	Change in EPC levels before and after 6-month exercise training	No	CD34+ levels were lower in HF patients than controls. EPC levels did not increase after exercise training
Jie 2011 [41]	RCT	65	45 HF patients with LVEF <50% randomized to Erythropoietin (n=30) or standard therapy (n=15) for 1 year and 20 unmatched healthy controls	CD34+ CD34+VEGFR2+	Yes	EPC levels	Yes	Erythropoietin did not increase circulating nor cultured EPCs Lower circulating EPCs were associated with older age, lower haemoglobin, lower creatinine clearance and higher interleukin 6 Circulating but not cultured EPCs were lower than controls

Tousoulis 2011 [49]	RCT	60	HF patients with LVEF<40% NYHA class II-IV randomized to Rosuvastatin 10 mg (n=21), Allopurinol 300mg (n=21) or placebo (n=18) for a month	CD34+ CD34+ VEGFR2+ CD34+CD133+ VEGFR2+	No	EPC levels	No	Just Rosuvastin increased EPC levels

* EPCs cultured according to the Hill protocol (replating non-adherent cells at 24-48 hours)

HF, heart failure; NYHA, New York Heart Association; BNP, b-type natriuretic peptide; VO_2, oxygen consumption; CAD, coronary artery disease; LVEF, left ventricular ejection fraction; CMP, cardiomyopathy; RCT, randomized controlled trial.

CHAPTER VI

The work described in this chapter has been published in the Canadian Journal of Cardiology. (Alba et al. Can J Cardiol 2013; 29(6):664-671.)

Full title: Circulating pro-angiogenic progenitor cells independently predict functional capacity in heart failure patients

Short title: Circulating progenitor cells in heart failure

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BRIEF SUMMARY

Circulating pro-angiogenic progenitor cells (CPCs) are endothelial and hematopoietic progenitor cells involved in the process of vasculogenesis. In this cross-sectional study including 121 ambulatory heart failure patients, we measured CPCs as circulating CD34+VEGFR2+cells and early outgrowth colony forming units (EO-CFUs). CD34+VEGFR2+cells were independently inversely associated with peak VO_2 while early outgrowth colony forming units (EO-CFUs) showed a positive association with peak VO_2. Cultured EO-CFUs represent functional capacity and vasculogenesis potential while CD34+VEGFR2+cells represent endothelial damage.

ABSTRACT

Background: Endothelial dysfunction is as an important underlying mechanism in the pathophysiology of heart failure (HF). Circulating pro-angiogenic progenitor cells (CPCs) are endothelial and hematopoietic progenitor cells involved in the process of vasculogenesis repairing damaged and dysfunctional endothelium. Our aim was to evaluate whether an independent association exists between CPCs and functional capacity in HF patients.

Methods: This cross-sectional study included 121 ambulatory HF patients with reduced left ventricular ejection fraction seen at a single institution. We analysed the association between CPCs, measured as circulating CD34+VEGFR2+cells and early outgrowth colony forming units (EO-CFUs), and patients' functional capacity measured as peak oxygen consumption (VO_2).

Results: The mean age was 55 ± 11 years; 96 patients (79%) were male. Forty-three (36%) patients had ischemic cardiomyopathy. Patients were on optimal HF therapy (96% on beta-blockers, 91% on renin-angiotensin inhibitors and 60% had an ICD implanted). In univariate analyses, CD34+VEGFR2+cells were inversely associated with peak VO_2 ($p=0.02$) while EO-CFUs showed a positive association with peak VO_2 ($p<0.01$). These associations persisted after adjusting for sex, NYHA class, body mass index, diabetes, cardiac resynchronization therapy, ischemic cardiomyopathy and b-type natriuretic peptide levels.

Conclusions: Cultured EO-CFUs may represent a measure of functional capacity and vasculogenesis potential while CD34+VEGFR2+cells represent the mobilized cells in

response to endothelial damage. Our study suggests that lower EO-CFUs (worse cell function) and higher CD34+VEGFR2+cells are associated with poorer functional capacity.

Key words: cardiac failure, prognosis, exercise testing, cells.

INTRODUCTION

Until the 1990s, it was commonly accepted that postnatal vasculogenesis occurred exclusively through the local outgrowth of pre-existing vessels by means of expansion of mature endothelial cells in response to angiogenic growth factors. However, certain peripherally circulating cell populations are believed to have endothelial reparative and angiogenic properties permitting adult vasculogenesis (1). The process of vasculogenesis is complex and not totally understood yet. Many different cells with different functions are involved in this process. According to functional assays and cell markers, two main types of cells have been described as playing important roles in adult vasculogenesis. Endothelial progenitor cells capable of forming vessels and pro-angiogenic hematopoietic progenitor cells promoting the process of angiogenesis probably via paracrine mechanisms lacking of own capacity of vessels formation (2). No specific cell markers have been described to distinguish endothelial progenitor cells from other cells (3,4). Pro-angiogenic hematopoietic cells may be derived from circulating blood cells co-expressing CD34, VEGFR2 and/or CD133 antigens and early-outgrowth (EO) cultured cells derived from peripheral blood mononuclear cells (also called EO-colony forming units (CFU)) (3). Many studies have analyzed the role of pro-angiogenic progenitor cells in patients with cardiovascular diseases in which endothelial dysfunction or damage is involved in disease development and progression. Endothelial dysfunction has been identified as an important underlying mechanism in the pathophysiology of heart failure (HF) (5-6) associated with disease severity and mortality irrespective of the presence of coronary artery disease (CAD) (7).

Studies analyzing the association between pro-angiogenic progenitor cells and disease severity in HF patients have yielded inconsistent results (8). Valgimigli et al (9) conducted a cross-sectional study evaluating the role of CD34+ cells, CD34+VEGFR2+ cells and EO-CFU in stable HF patients. They observed that levels of CD34+ and CD34+VEGFR2+ cells and EO-CFU were higher in NYHA class I or II patients than healthy controls, while NYHA class III or IV patients had lower cell levels. Pellicia et al (10) et al, however, reported that in a group of 68 patients with stable CAD, CD34+CD133+ cells were inversely associated with left ventricular ejection fraction (LVEF). Reasons for these discrepant results may include differences in study populations, the type of cells assessed and the use of unadjusted analysis.

Peak oxygen consumption (peak VO_2) measured by cardiopulmonary exercise stress testing is commonly used to evaluate the severity of HF. Peak VO_2 is a powerful prognostic factor that has proved useful in monitoring HF progression, in particular helping to select patients for cardiac transplantation. In the present study, we addressed a possible independent association between circulating progenitor cell (CPC) levels, measured as circulating cells co-expressing CD34, VEGFR2 and/or CD133 antigens and EO-CFU, and peak VO_2 in HF patients with reduced LVEF.

METHODS

Study population

In this cross-sectional study, we included 121 consecutive consenting ambulatory HF patients seen at the Toronto General Hospital from July 2010 to April 2011. The

institutional review board approved this study. Inclusion criteria were LVEF <40%, no HF hospital admission for a period of 2 months and capable of performing a cardiopulmonary study. Patients with cancer within 5 years before enrolment and patients with active inflammatory conditions were excluded as these diseases may affect CPC levels.

Blood sampling

Two venous blood samples were collected at the time patients came to clinic. One was collected using a BD Vacutainer CPT tubes (Becton Dickinson, San Jose, CA) and was used to measure CPC levels. This sample was stored at room temperature and processed within 2 hours of collection. The other sample was taken using EDTA tubes and immediately placed on ice and centrifuged within 1 hour of collection at 4000 rpm (1400 g) for 20 min; plasma was harvested and stored into aliquots at -80°C until batch analysis. This sample was used to measure circulating cytokines, including tumor necrosis factor alfa (TNF-α), VEGF-A and interleukin-6.

Peripheral blood mononuclear cell isolation

Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-density gradient centrifugation. We washed recovered cells twice with phosphatebuffered saline (PBS) with 2% fetal bovine serum. We re-suspended the cells in CFU-Hill Liquid Medium (StemCell Technologies), count and then used them for various assays.

Early-outgrowth colony-forming units

After cells were re-suspended in CFU-Hill Liquid Medium, we plated 5 million cells on fibronectin coated 6-well plates (Biocoat, Becton Dickinson Labware) for 24 to

48 hours days to remove mature endothelial cells and platelets (11). One million non-adherent cells were then re-plated on a fibronectin-coated-24-well plates (Biocoat, Becton Dickinson Labware) using 4 wells per patient. We quantified colonies 3 days later. A colony was defined as a central core of round cells with radiating elongated spindle-like cells at the periphery. One colony represented one EO-CFU. We expressed values as mean EO-CFU per well. Colony counting was performed by 2 independent investigators blinded to patients' data. Inter-rater reliability assessed on 30 patients using G theory and considering the variances associated with raters, patients and wells was 0.88.

Circulating pro-angiogenic progenitor cells

Circulating pro-angiogenic progenitor cells were identified from isolated PBMC and counted through triple-staining with monoclonal antibodies: Fluoresceinisothiocyanate (FITC)-conjugated anti-CD34 (Miltenyi Biotec, Auburn, CA), R-phycoerythrin-conjugated anti-VEGFR2 (Myltenyi Biotec, Auburn, CA), and allophicocyanin-conjugated anti-CD133/1 (Myltenyi Biotec, Auburn, CA). Supplemental Methods (S1) explains the protocol utilized. Results were calculated as percentages of CD34+ cells that co-expressed CD133 and/or VEGFR2 antigens on their surface, and finally, their number were expressed as number of cells/1 million PBMC for each patient. A single blinded investigator to patients' characteristics performed cell counting. The intra-assay reliability obtained by intra-class correlation on 20 patients was 0.94.

Peak oxygen consumption

All patients underwent a cardiopulmonary exercise stress testing (CPET); 106 (88%) patients at the time of blood extraction and the rest within 3 months prior to blood

extraction. CPET was performed by an experienced technician using a commercially available cycle ergometer (Lode MedGraphics, Minneapolis, MN) and a metabolic cart (MedGraphics CardiO2-Ultima, Minneapolis, MN) in most patients (96%). The work rate was increased using a continuous ramp protocol of 10 watts/minute in all patients. Breath by breath analysis of the expired gases was performed. We attempted to reach a respiratory exchange ratio (RER) value of at least 1.1 in all patients. The average of the VO_2 levels obtained during the mid 5 breaths of the last 7 breaths was used as a measure of peak VO_2.

Other variables

We collected clinical and laboratory data, including demographic characteristics (age, sex, race), co-morbidities (diabetes, hypertension, smoking, peripheral vascular disease, chronic lung disease), HF history (underlying cause, last HF hospital admission, medications) and physical examination (body mass index (BMI), current NYHA class, heart rate and blood pressure at rest). Laboratory values included hemoglobin, leucocytes, lymphocytes, electrolytes, BUN (blood urea nitrogen), serum creatinine, total cholesterol, uric acid, BNP (b-type natriuretic peptide) and C-reactive protein.

Statistical analysis

In describing the study population, categorical variables were represented as proportions and continuous variables were summarised by their means and standard deviations (SD). We performed multivariable linear regression to evaluate whether there was an independent association between CPC levels and peak VO_2. Independent variables were selected according to their bivariate correlations. Ten factors (sex, diabetes,

ischemic CMP, LVEF, CRT, NYHA, BMI, creatinine, haemoglobin and BNP) that were significantly correlated with peak VO_2 ($p<0.05$) were entered in a multivariable regression; stepwise backward elimination was applied, in which the least significant variables were sequentially removed according to a pre-specified p-value of ≥ 0.1. After eliminating all non-significant variables, CD34+VEGFR2+cell levels and EO-CFU levels were then simultaneously entered in a block into the model. Thirty-eight (31%) patients had undetectable levels of CD34+VEGFR2+CD133+cells. This limits the ability and use of CD34+VEGFR2+CD133+cells to differentiate patients. CD34+CD133+cells were not significantly associated with peak VO_2. Therefore, CD34+VEGFR2+cells were used in these analyses. We assessed model assumptions through the analysis of residuals, including conditional normality of errors and homoscedasticity (constant variance), which were fulfilled. We determined confidence intervals of estimated coefficients and statistical significance using bootstrapping with 1000 replications. Estimated β coefficients express the differences of peak VO_2 between groups in the case of a binary predictor variable and the change of peak VO_2 associated with one-unit change of the predictor in the case of a continuous predictor variable.

Subsequently, we identified factors associated with CD34+VEGFR2+cells and EO-CFU levels using univariate negative binomial regression models. Variables that were associated with the dependent variable with p value < 0.2 were entered in a multivariable negative binomial regression. The association between cell levels and predictors was expressed in percentage change of cell levels between groups in the case of a binary predictor variable and the percentage change of CD34+VEGFR2 cell or EO-CFU levels

associated with one-unit change of the predictor in the case of a continuous variable. The percentage change was calculated by exponentiation of the estimated coefficient. The goodness-of-fit of model was assessed after removing a variable by backward selection and compared using likelihood ratio test. A p value < 0.05 was considered to indicate a significant change in the model fit.

We calculated the sample size based on the use of linear regression as our primary statistical analysis. The expected correlation (r) between CPC levels and peak VO2 was between 0.4 and 0.6 (9); setting 2-sided $\alpha = 0.05$ and $\beta = 0.2$, the minimum required sample size was 60 patients. We doubled the sample size to ensure a sufficient number of patients to permit multivariable analysis with a maximum of 10 to 12 predictor variables without risk of over-fitting.

Statistical analysis was performed using Stata 12 (College Station, TX).

RESULTS

Baseline characteristics

The mean age was 56 years and SD of 11 years (minimum 24 and maximum of 78 years) and 96 (79%) patients were male (Table 1). Overall, 81% of the patients were NYHA class II to IV, 96% were using β-blockers, 91% inhibitors of the renin-angiotensin system and 60% an ICD/CRT (internal cardiac defibrillator/cardiac resynchronization therapy). Causes of cardiomyopathy were idiopathic in 46%, ischemic in 35%, familial dilated cardiomyopathy (CMP) in 4%, arrhythmogenic right ventricular dysplasia in 4%, hypertrophic CMP in 6%, peri-partum and valvular CMP. The mean peak VO2 was 14

and SD of 4 ml/kg/min. The median level of circulating CD34+VEGFR2+cells and EO-CFU were 10 cells/million PBMN (inter-quartile range, 3 to 22 cells/million PBMC) and 10 colonies/well (inter-quartile range, 4 to 26 colonies/well), respectively.

Association between levels of circulating pro-angiogenic cell and peak VO_2

Table 2 presents variables associated with peak VO_2. The final model includes female sex, diabetes, ischemic cardiomyopathy, higher BMI, use of CRT, worse NYHA class and higher BNP levels as independent predictors of lower peak VO_2.

The univariable analyses demonstrated that CD34+VEGFR2+cells were inversely correlated with peak VO2 while EO-CFU were positively correlated ($p<0.05$). The statistically significant association between CD34+VEGFR2+cells and EO-CFU with peak VO_2 persisted after adjusting for the predictors cited in Table 2, and showed that an decrease of 10 CD34+VEGFR2+cells was independently associated with an increase in peak VO_2 of 0.28 ml/kg/min. Conversely, increase of 10 EO-CFU was independently associated with a change in peak VO_2 of 0.33 ml/kg/min. Table 3 and Figure 1 show these results. CD34+VEGFR2+cells and EO-CFU were not significantly associated.

Supplemental Table S1 shows the univariable analysis of factors associated with CPC levels. The presence of dyslipidemia, ischemic cardiomyopathy as the underlying aetiology of HF and use of statins were significantly associated with higher levels of CD34+VEGFR2+cells. In multivariate analysis, only ischemic cardiomyopathy and dyslipidemia were independently associated with circulating CD34+VEGFR2+cells. Patients with ischemic cardiomyopathy had a 55% decrease in circulating CD34+VEGFR2+cells when compared to patients with non-ischemic cardiomyopathy

(predicted mean of 26 cells/million PBMC and 14 cells/million PBMC, respectively) (Figure 2). Patients with dyslipidemia had a 35% increase in CD34+VEGFR2+cells when compared to patients without dyslipidemia (predicted mean of 22 and 13 cells/million PBMC, respectively).

Factors that were significantly associated with increased EO-CFU by univariable analysis were non-ischemic cardiomyopathy and VEGF-A levels. In multivariable analysis, levels of VEGF-A and non-ischemic cardiomyopathy persisted as independently associated factors. For an increase of 10-unit change in VEGF levels, EO-CFU decreased by 28%. In addition, patients with non-ischemic underlying cardiomyopathy have 45% higher levels of EO-CFU than patients with ischemic cardiomyopathy. The predicted median of EO-CFU was 14 units/well in patients with ischemic cardiomyopathy and 20 units/well in patients with non-ischemic cardiomyopathy (Figure 2).

DISCUSSION

In this study, we observed that higher levels of CD34+VEGFR2+cells and lower EO-CFU levels were factors independently associated with poorer functional capacity in HF patients, after adjusting for sex, BMI, diabetes, cause of cardiomyopathy, NYHA class, use of CRT and BNP levels. Experimental data have confirmed the key role of circulating progenitor cells in adult vascular health with the capacity of repairing lost and dysfunctional endothelium. A variety of growth factors and cytokines released in response to tissue ischemia are associated with the number and function of CPCs. Inflammatory activation, endothelial dysfunction and endothelial damage are important in

the pathogenesis of HF, contributing to cardiac remodelling and peripheral vascular disturbances. Endothelial dysfunction contributes to elevated peripheral vascular resistance, limits blood flow to the periphery and determines a perfusion–regulation mismatch resulting in organ ischemia, which leads to vascular damage, exacerbating the original problem. Endothelial dysfunction has been associated with HF severity and mortality (7).

Circulating cells co-expressing CD34, VEGFR2 and/or CD133 antigens increase after several stimuli of endothelial injury including surgery (12), myocardial infarction (13) and burn injury (14); they migrate to areas of ischemia and participate in the process of vasculogenesis (15,16). In HF, endothelial dysfunction and tissue ischemia is more manifest in patients with more severe HF stage (7). Our finding that CD34+VEGFR2+cells were higher in patients with worse functional capacity as measured by peak VO_2 support the pathophysiological mechanism of progenitor cell mobilization associated with endothelial dysfunction and damage. In our study, CD34+VEGFR2+ cells were significantly higher in patients with ischemic cardiomyopathy, probably consistent with a higher burden of vascular damage and endothelial dysfunction. Importantly, the association between CD34+VEGFR2+ cells and patients functional capacity (peak VO_2) persisted after adjusting for disease aetiology.

Studies analyzing the role of CPC in HF patients have shown inconsistent results. Similar to our results, levels of circulating CD34+VEGFR2+cells were higher in a group of 68 patients with stable CAD and reduced LVEF of whom 55% were NYHA class III-IV versus CAD patients with preserved LVEF (10). Other studies (17,18) measuring CPC

in stable HF patients have found no association between CD34+ cells and NYHA class. This may be related to small sample sizes and low statistical power.

In contrast, Valgimigli et al (9) measured CD34+ cells in 41 patients with congestive HF and found that NYHA class I or II HF outpatients had higher levels of CD34+ cells than healthy controls, while NYHA class III or IV patients had lower levels of CPCs. They reported no association between circulating CPCs measured as CD34+VEGFR2+CD133+ cells and peak VO_2. Importantly, in Valgimigli study, statins treatment was discontinued for a minimum of 3 weeks prior to blood extraction in all patients. Statins increase the levels of circulating progenitor cells in HF patients as demonstrated in a randomized control trial (19). In our study, patients did not stop any medication and in fact, we found that statin treatment was associated with higher CD34+VEGFR2+ cell levels in univariate analysis. This difference in medication use, the small sample size and the use of unadjusted analysis in the Valglimigli et al's study may partly explain the discrepant results found between studies.

Our study suggested an inverse association between circulating CD34+VEGFR2+ cells and HF severity as assessed by peak VO_2, and we hypothesize that the potential link may be higher CD34+VEGFR2+ cell mobilization in response to more pronounced endothelial injury and dysfunction.

Early-outgrowth CFU represents a measure of functional capacity of circulating progenitor cells. Lower levels of EO-CFU are associated with higher cardiovascular risk (11) and disease severity (20-22). In our study, higher levels of EO-CFU were associated with patients' functional capacity independently of other clinical factors and levels of

CD34+VEGFR2+cells. Other studies have analyzed the role of EO- CFU in patients with HF. Consistent with our results, Valgimigli et al (9) found that patients with NYHA class III-IV had lower EO- CFU than NYHA class I-II patients. These results support the hypothesis that higher pro-angiogenic progenitor cell function manifested as higher EO- CFU levels suggest increased potential for endothelial repair, which could play a protective role against disease progression in HF patients. Conversely, the single study (23) that has evaluated the association between EO- CFU and mortality in HF patients reported that higher levels of EO- CFU were associated with higher risk of all-cause mortality. However, important statistical limitations in the analysis of this study make its results questionable.

We found that EO- CFU levels were lower in patients with an ischemic cause of cardiomyopathy, consistent with the association of higher EO- CFU levels and better vascular health described in different population of patients with cardiovascular diseases. Higher levels of VEGF-A were significantly associated with lower EO- CFU. VEGF production can be induced in many cells that are under hypoxic conditions. In vitro, VEGF-A stimulates endothelial cell mitogenesis and cell migration by binding VEGFR1 and VEGFR2 acting as a promoting vasculogenesis factor (24). EO- CFU mainly represents hematopoietic cells that produce large quantities of angiogenic cytokines and enhance vessel formation both in vitro and in vivo (3). This negative association has also been describe in vitro (4) and may represent a regulatory mechanism between cells and cytokines involved in the process of vasculogenesis. Further research in basic science may help to elucidate these interactions.

Similar to our results, some studies (8,9) have reported no correlation between CD34+VEGFR2+cells and EO- CFU, suggesting that these cells represent different cell populations, leading some researchers to propose renaming these cells (3). Although the purpose of this study was not to clarify the origin of these cells, these results add to the increasing evidence that these cells are dissimilar in nature. An elegantly designed study by Lin et al (25) explored the origin of these cells in 4 patients who underwent a sex-mismatched bone marrow transplant (making possible the differentiation of circulating cells with donor or recipient genotype). They found that more than 95% of circulating CD34+VEGFR2+cells had a recipient genotype while the expanded culture of PBMC mostly displayed a donor genotype. Based on these results, they concluded that most of the circulating CD34+VEGFR2+cells are vessel derived while cultured cells are mainly bone marrow derived.

Study limitations

Our study's cross-sectional design limits the assessment of a temporal association between circulating pro-angiogenic progenitor cell levels and patients' functional capacity. Unlikely most if the previous studies, we used peak VO_2 as an indicator of disease severity, which is commonly accepted as an influential and useful prognostic factor in HF patients, specifically helping to select patients for advanced therapeutic options. Although peak VO_2 is a potent prognostic marker in patients with HF, studies analyzing HF outcomes, including death, or need for cardiac transplantation or mechanical circulatory support, may provide stronger evidence about the potential role of these cells as a prognostic marker in HF patients.

Even though this study is the largest analyzing simultaneously CD34+VEGFR2+ cells and EO-CFU in HF patients and the unique study using adjusted analysis, sample size limited the number of predictors in the model to predict peak VO_2 and required us to use a backward regression algorithm. While this may increase the risk of biased results, the introduction of levels of CC34+VEGFR2+ cells and EO-CFU in a second block and the use of bootstrapping to estimate coefficients and measures of uncertainty minimize the risk of bias in the estimated association between CPC levels and peak VO_2.

CONCLUSIONS

Higher levels of CD34+VEGFR2+cells and lower levels of EO-CFU were independently associated with functional capacity as measured by peak VO_2 in HF patients after adjusting for other important clinical variables. Given that CD34+VEGFR2+cells increase in response to tissue ischemia and endothelial damage and that EO-CFU express the function of these cells and potential vasculogenesis properties, HF patients with higher pro-angiogenic cell mobilization and worse function present with more severe disease as assessed by patients' functional capacity. This evidence suggests that pro-angiogenic progenitor cells are possibly involved in the pathophysiologic process of HF constituting a promising prognostic factor and potential therapeutic target.

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TABLES

Table 1: Baseline characteristics

Variable	**n= 121** mean ± SD / n (%)
Age (years)	56 ± 11
Female sex	25 (21)
Black race	6 (5)
Hypertension	49 (41)
Diabetes	26 (21)
Dyslipidemia	71 (59)
Body mass index (kg/m^2)	29 ± 7
Ischemic CMP	43 (35)
β-blockers	116 (96)
ACE inhibitors or ARB	110 (91)
Spironolactone	59 (49)
Statins	70 (58)
Digoxin	46 (38)
Furosemide	83 (69)
Furosemide (mg/day) *	100 ± 70
ICD	73 (60)
CRT	37 (30)
NYHA class I/II/III/IV	18(19)/57(47)/34(33)/2(1)
Peak VO_2 (ml/min/kg)	14 ± 4
Systolic blood pressure at rest (mmHg)	102 ± 15
Heart rate at rest (bpm)	68 ± 11
LV ejection fraction (%)	29 ± 8
QRS (msec)	138 ± 40

Table 1. Continued.

Variable	**n= 121** mean ± SD / n (%)
Hemoglobin (g/dl)	14 ± 1.4
Creatinine (mg/dl)	1.2 ± 0.45
BUN (mg/dl)	26 ± 11
Sodium (meq/l)	138 ± 3
BNP (Median ± IQR) (pg/ml)	188 (379)
Cholesterol (mg/dl)	170 ± 46
C-reactive protein (mg/dl)	2 ± 3
Interleukin 6 (pg/ml)	2.8 (3.4)
TNF-α (pg/ml)	1.7 (3)
VEGF-A (pg/ml)	32 ± 12

* Including just 83 patients who were on Furosemide

ACEI, angiotensin-converter enzyme; ARB, angiotensin II receptor blockers, ICD, internal cardiac defibrillator, CRT, cardiac resynchronization therapy, NYHA, New York Heart Association; VO_2, oxygen consumption; LV, ejection fraction, BUN, blood urea nitrogen; BNP, b-type natriuretic peptide; IQR, inter-quartile range; TNF-α, tumor necrosis factor alpha, VEGF, vascular endothelial growth factor.

Table 2: Univariable and multivariable regression analysis between relevant clinical variables and peak oxygen consumption

Variable	**Univariable analysis**		**Multivariable analysis**	
	β coefficient	**P value**	**β coefficient**	**P value**
Age (per 10-year increase)	- 0.52	0.10		
Female sex	-2.05	0.02	-2.3	0.001
Diabetes	-4.03	<0.001	-2	0.006
BMI (per 1-kg/m^2 increase)	- 0.12	0.02	-1.13	0.07
Ischemic CMP	- 1.8	0.02	-0.14	0.002
CRT	- 2.6	0.005	-1.8	0.005
NYHA class (per class increase)	- 2.9	<0.001	-1.6	<0.001
LV ejection fraction (per 10-% increase)	1.2	0.001		
Hemoglobin (per 10-g/dl increase)	0.7	0.006		
Creatinine (per 10-mg/dl increase)	- 0.3	0.001		
BNP (per log-unit change)	- 0.55	<0.001	-0.49	0.04
Cholesterol (per-40 mg/dl increase)	0.6	0.05		

CRT, cardiac resynchronization therapy, NYHA, New York Heart Association; LV, ejection fraction, BNP, b-type natriuretic peptide.

Table 3: Analysis to evaluate the independent predictive value of CD34+VEGFR2+ cells and early-outgrowth colony forming units (EO-CFU)

Variable	**Observed β coefficient**	**Bootstrap estimates**		
		Averaged β coefficient	**95% confidence interval**	**p**
Intercept [#]	19	21	16 – 22	<0.001
Female sex	-2.02	-2.03	-3.2 – -0.87	0.001
Diabetes	-1.97	-1.97	-3.1 – -0.88	<0.001
Ischemic CMP	-0.54	-0.56	-1.6 – 0.56	0.33
BMI (per kg/m^2)	-0.12	-0.12	-0.21 – -0.03	0.009
CRT	-1.94	-1.96	-3 – -0.89	<0.001
NYHA class	-1.6	-1.58	-2.4 – -0.80	<0.001
BNP ~	-0.47	-0.47	-1 – 0.07	0.09
CD34+VEGFR2+ (per 10 cells/million PBMC)	-0.28	-0.28	-0.54 – -0.1	0.03
EO-CFU (per 10 units/well)	0.32	0.33	0.1 – 0.58	0.02

[#] The intercept represents the predicted value of peak VO2 for a non-diabetic male patient, with a BMI of 20 kg/m^2 and with NYHA class I non-ischemic cardiomyopathy and no CRT and low levels of BNP and zero level of both circulating progenitor cells. Estimated β coefficients express the differences of peak VO_2 between groups in the case of a binary predictor variable and the change of peak VO_2 associated with one-unit change of the predictor in the case of a continuous predictor variable.

~ BNP levels were log transformed because the distribution was markedly positively skewed. CMP, cardiomyopathy; BMI, body mass index; CRT, cardiac resynchronization therapy, NYHA, New York Heart Association; BNP, b-type natriuretic peptide.

LEGENDS OF FIGURES

Figure 1. Scatter plots picturing the association between CD34+VEGFR2+ cells (panel A) and EO-EPC (panel B) and peak oxygen consumption.

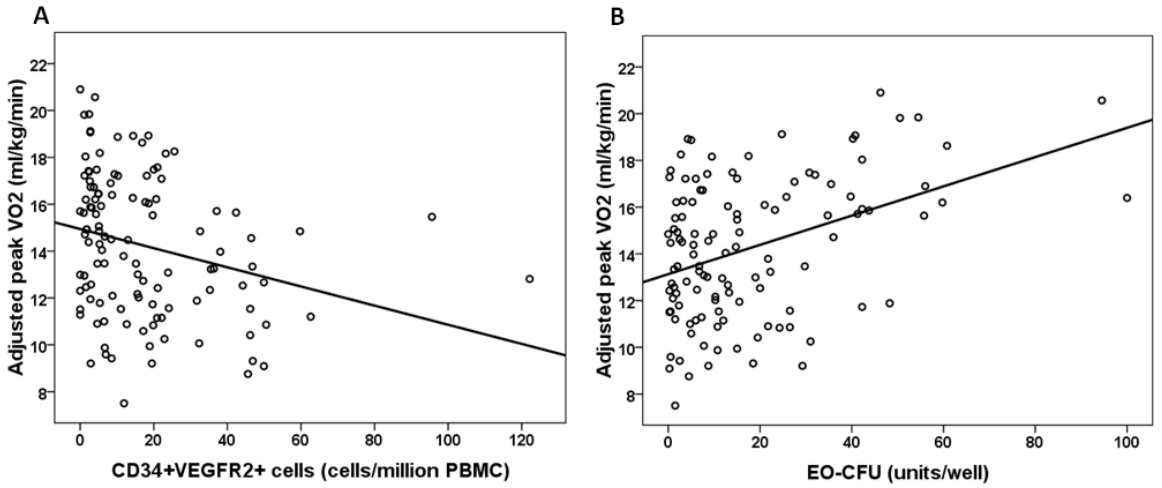
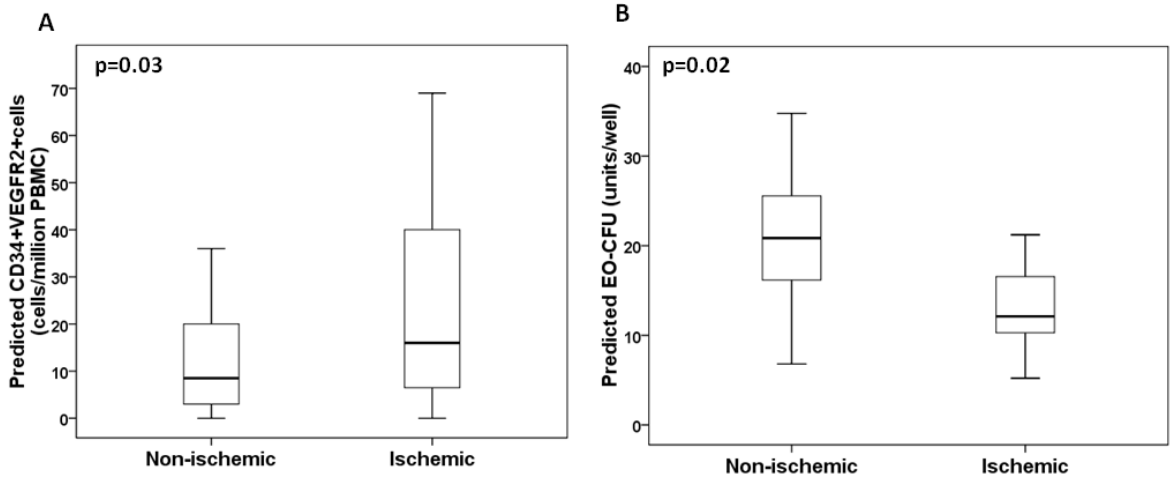


Figure 2. Predicted levels of CD34+VEGFR2+ cells (panel A) and EO-EPC (panel B) according to the underlying cause of cardiomyopathy (panel B).

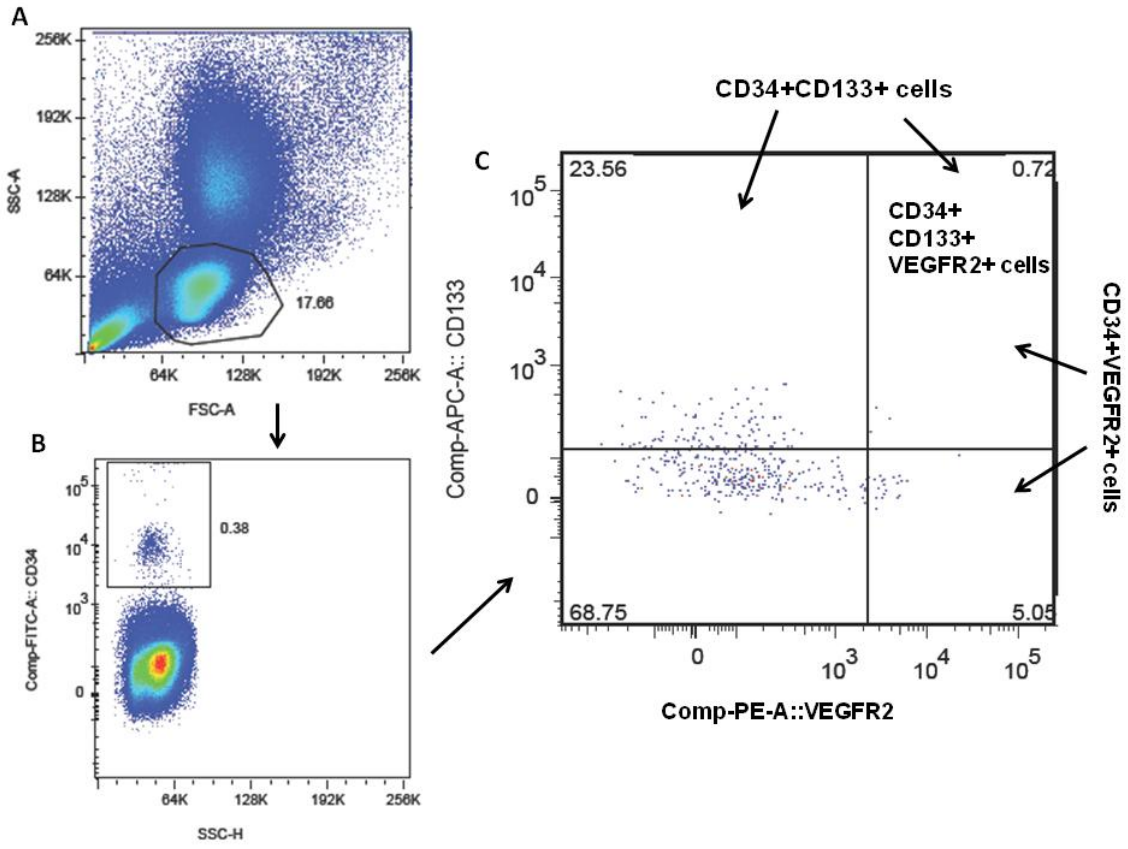


S1: Supplemental Methods

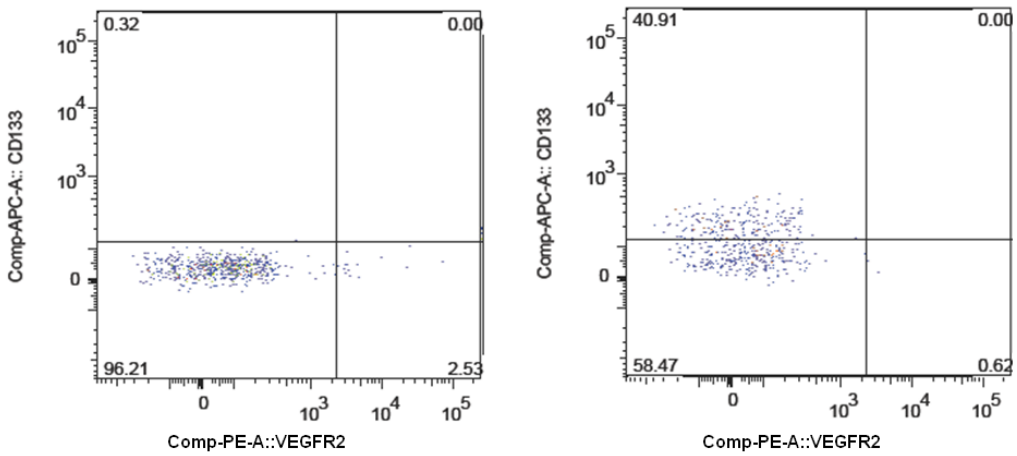
Measure of circulating pro-angiogenic progenitor cells by flow cytometry

Circulating pro-angiogenic progenitor cells were identified and counted through triple-staining with monoclonal antibodies: Fluoresceinisothiocyanate (FITC)-conjugated anti-CD34 (Miltenyi Biotec, Auburn, CA), R-phycoerythrin-conjugated anti-VEGFR2 (Myltenyi Biotec, Auburn, CA), and allophicocyanin-conjugated anti-CD133/1 (Myltenyi Biotec, Auburn, CA). After cells were re-suspended in CFU-Hill Liquid Medium (StemCell Technologies), 2 million cells were washed with flow buffer, containing PBS, 0.5% bovine serum albumin (BSA) and 2 mM EDTA, then re-suspended and treated for 15 minutes with a FcR blocking agent (Myltenyi Biotec, Auburn, CA) to decrease unspecific binding and finally treated with the antibodies for 25 minutes. Isotype-identical antibodies against human cells (Myltenyi Biotec, Auburn, CA) and samples with combinations of just 2 of the mentioned antibodies served as controls. We processed the samples using the LSR II Flow Cytometer System (Becton Dickinson, San Jose, CA), and analyzed them using FACS Diva software (Becton Dickinson, San Jose, CA). We determined 2 different cell sub-populations with the phenotypes CD34+VEGFR2+ and CD34+VEGFR2+CD133+. We collected a minimum of 500000 events per sample. After excluding cellular debris in a forward scatter/side scatter plot according to their size and granularity and gating on CD34+ cells, cells were further characterized as CD34+ cells co-expressing CD133 and/or VEGFR2 antigens. The immunofluorescence cutoff was set based on controls and kept constant in all scatter plots.

Figure picturing the protocol used to measure circulating progenitor cells by triple staining with CD34, VEGFR2 and CD133 antibodies.



The immunofluorescence cutoff was set based on controls and kept constant in all scatter plots. Sample with combinations of just 2 of the mentioned antibodies served as controls (CD34 and CD133 in right panel and CD34 and VEGFR2 in left panel).


0.32
0.00
96.21
2.53
Comp-APC-A:: CD133
Comp-PE-A::VEGFR2
40.91
0.00
58.47
0.62
Comp-APC-A:: CD133
Comp-PE-A::VEGFR2

SUPPLEMENTAL TABLES

Supplemental Table S1. Univariable analysis of factors associated with peak oxygen consumption.

Variable	β-coefficient	p-value
Age (per 10-year increase)	-0.52	0.10
Female sex	-2.05	0.02
Black race	-2.4	0.16
Hypertension	-0.7	0.31
Diabetes	-4.03	<0.001
Dyslipidemia	-0.11	0.89
Body mass index (per 1-kg/m^2 increase)	-0.12	0.02
Ischemic cardiomyopathy	-1.8	0.02
β-blockers	-0.35	0.85
ACE inhibitors or ARB	-0.17	0.84
Spironolactone	-1.2	0.15
Statins	-1.5	0.04
Cardiac resynchronization therapy	-2.6	0.001
NYHA class	-2.9	<0.001
Systolic blood pressure at rest (per 10-mmHg increase)	0.4	0.12
Heart rate at rest (per 10-bpm)	-0.38	0.29
LV ejection fraction (per 10-% increase)	1.2	0.001
Hemoglobin (per 1-g/L increase)	0.7	0.006
Creatinine (per 10-µmol/L increase)	-0.3	0.001
Sodium (per 10-meq/l)	1.4	0.26
BNP (per log unit) (pg/ml)	-0.55	<0.001
Cholesterol (1-mmol/L increase)	0.6	0.05
C-reactive protein (per log unit)	-0.3	0.11

ACEI, angiotensin-converter enzyme; ARB, angiotensin II receptor blockers; NYHA, New York Heart Association; LV, ejection fraction, BUN, blood urea nitrogen; BNP, b-type natriuretic peptide.

Supplemental Table S2. Univariable analysis of factors associated with levels of CD34+VEGFR2+ cells and early-outgrowth colony-forming units (EO-CFU)

Variable	**CD34+VEGFR2+cells**		**EO-CFU**	
	Relative change	**p**	**Relative change**	**p**
Age (per year)	1.2%	0.16	-0.2%	0.86
Male sex	0.4%	0.98	31%	0.28
BMI (per kg/m^2)	1.7%	0.32	-1.2%	0.38
HTN	8.4%	0.67	-6%	0.76
Diabetes	34%	0.19	-16%	0.44
Dyslipidemia	68%	0.006	31%	0.16
Ischemic CMP	62%	0.005	-47%	0.02
Systolic BP (per mmHg)	0.4%	0.53	0.6%	0.37
NYHA class (per class)	0.5%	0.97	-13%	0.27
B-Blockers	3.4%	0.94	50%	0.31
ACEI	-18%	0.38	-6%	0.75
Statins	72%	0.005	-4%	0.84
ICD	-0.17	0.39	11%	0.62
CRT	0.05	0.79	0.9%	0.96
LV EF (per %)	-0.3%	0.74	13%	0.21
Hemoglobin (per g/L)	0.4	0.44	13%	0.08
Creatinine (per μmol/L)	0%	0.96	0.2%	0.39
Uric acid (per μmol/L)	0%	0.48	0.1%	0.93
Cholesterol (per 1 mmol/L)	0.1%	0.99	8%	0.34
BNP (per log unit)	-3%	0.71	-6%	0.37
C-reactive protein (per mg/l)	-0.9%	0.57	-1%	0.47
Interleukin 6 (per ng/ml)	-1.5%	0.42	-2%	0.25
TNF-α (per ng/ml)	-2.6%	0.49	2%	0.59
VEGF-A (per 10 ng/ml)	11%	0.29	-35%	<0.001

BMI, body mass index; HTN, hypertension; CMP, cardiomyopathy; BP, blood pressure; NYHA, New York Heart Association; ACEI, angiotensin-converter enzyme; ICD, internal cardiac defibrillator, CRT, cardiac resynchronization therapy; LVEF, left ventricular ejection fraction, TNF-α, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor.

CHAPTER VII

The work described in this chapter has been accepted for publication in the Canadian Journal of Cardiology. (Alba et al. Can J Cardiol 2013; in press)

Full title: Changes in circulating progenitor cells are associated with outcome in heart failure patients: A longitudinal study

Short title: Circulating progenitor cells in heart failure

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BRIEF SUMMARY

Circulating pro-angiogenic progenitor cells (CPCs) are involved in the process of vasculogenesis. This longitudinal study included 156 ambulatory heart failure patients, with serial CPC measurements as circulating CD34+VEGFR2+cells and early outgrowth colony forming units (EO-CFUs) during 2 years. Higher EO-CFUs and lower CD34+VEGFR2+cells were associated with improvements in peak VO_2 in non-diabetic HF patients. In diabetics, lower EO-CFUs were associated with better peak VO_2 suggesting a differential behaviour. Higher EO-CFUs were associated with reduced mortality.

ABSTRACT

Background: Circulating progenitor cells (CPCs) are involved in the process of endothelial repair and are a prognostic factor in cardiovascular diseases. We evaluated the association between serial measurements of CPCs and functional capacity and outcomes in heart failure (HF) patients.

Methods: We included 156 consecutive consenting ambulatory HF patients (LVEF<40%). We evaluated CPCs and functional capacity every 6 months for up to 2 years. CPCs were measured as early-outgrowth colony-forming units (EO-CFU) and circulating CD34+, VEGFR2+ and/or CD133+cells. Functional capacity was measured as peak oxygen consumption (peak VO_2). We recorded mortality, HF hospital admissions and a composite outcome of death, transplant and ventricular assist device implantation.

Results: The mean age was 55 ± 15 years; 31patients were female. A decrease in CD34+VEGFR2+cells was independently associated with increased functional capacity; a 10-cell decrease in CD34+VEGFR2+cells was associated with an increase of 0.2 ml/kg/min in peak VO_2 ($p<0.05$). We found an interaction effect ($p=0.02$) between EO-CFUs and diabetes: in non-diabetics a 10-EO-CFU increase was independently associated with an increased peak VO_2 of 0.28 ml/kg/min ($p=0.01$), and in diabetics a decrease in EO-CFUs was associated with an increased peak VO_2 ($p<0.05$). Higher EO-CFUs were associated with reduced mortality (HR 0.25, 95%CI0.09-0.69).

Conclusions: We noted differential relations between CPCs and outcomes in diabetic versus non-diabetic patients. Higher EO-CFUs and lower CD34+VEGFR2+cells were associated with improved functional capacity and reduced mortality in non-diabetic

patients. In diabetics, lower EO-CFUs were associated with improved functional capacity. The basis for these differences requires further examination in future studies.

Key words: Circulating progenitor cells; Heart failure; Exercise testing; Follow-up studies; mortality

INTRODUCTION

Heart failure (HF) is a common disorder associated with high mortality and persistent morbidity [1,2]. Patients' functional status and quality of life gradually decline as the reduced cardiac output does not satisfy the body's demands, resulting in symptoms of fatigue and dyspnea.

The pathophysiological basis of limited functional capacity has yet to be understood. Abnormalities in cardiac function are not sufficient to fully explain exercise intolerance in HF patients. Indices of resting ventricular function such as left ventricular ejection fraction (LVEF) correlate poorly with peak exercise capacity [3]. Peripheral limitations such as a reduced quality of skeletal musculature including pathological changes in muscle fibres, reduction in capillary density and impaired peripheral perfusion secondary to endothelial dysfunction have been identified as additional factors responsible for the impaired functional status of HF patients [4-6].

Endothelial dysfunction is primarily determined by the altered bioavailability of nitric oxide (NO) and increased oxidative stress [7]. Endothelial dysfunction leads to impaired vasodilation in the coronary, pulmonary, renal and peripheral vascular beds [8], and myocardial dysfunction [9]. In patients with HF, endothelial dysfunction is associated with disease severity and mortality irrespective of the presence of coronary artery disease [10,11]. Several therapies, with proven survival benefit, such as ACE inhibitors, β-blockers, spironolactone, nitrates when administered with hydralazine and exercise, improve endothelial function in patients with HF [12].

Circulating progenitor cells (CPCs) involved in the process of adult vasculogenesis and capable of repairing lost and dysfunctional endothelium have drawn increased interest. Based on functional assays and cell markers, two main types of cells have been identified as playing important roles in adult vasculogenesis: endothelial progenitor cells, which are cells capable of forming vessels, and pro-angiogenic hematopoietic progenitor cells, which promote the process of angiogenesis, probably via paracrine mechanisms [13]. Most studies evaluating the role of CPCs in HF have focused on this latter group of cells [14]. Pro-angiogenic hematopoietic cells may be identified from their co-expression of CD34, VEGFR2 and/or CD133 antigens or from early-outgrowth (EO) cultured cells derived from peripheral blood mononuclear cells (also called EO-colony forming units (CFU)) [13].

We recently demonstrated in a cross-sectional study including HF patients that higher levels of CD34+VEGFR2+cells and lower levels of EO-CFU were independently associated with functional capacity as measured by peak oxygen consumption (peak VO_2) [15]. Given that CD34+VEGFR2+cells increase in response to tissue ischemia and endothelial damage [13] and that EO-CFU represent the functional capacity of these cells and potential vasculogenesis properties [13], HF patients with higher pro-angiogenic cell mobilization and worse cell function have more severe disease as assessed by functional capacity.

The objective of the present study was to examine the independent association between changes in CPC levels and changes in functional capacity (peak VO_2) over time in adult patients with HF. In addition we explored whether this association differed based

on cardiovascular risk factors, such as age, sex, diabetes, hypertension, dyslipidemia and obesity. We also addressed the association between CPC levels and all-cause mortality, hospital admissions due to decompensated HF, and a composite end-point of death, urgent heart transplantation (HTx) and urgent ventricular assist device (VAD) implantation.

METHODS

Study population

In this prospective cohort study, we included 156 consecutive consenting ambulatory HF patients from July 2010 to January 2011. Of these patients, 121 were enrolled in our previous cross-sectional study [15]. At the time of enrolment, all patients had been seen at least once at the Toronto General Hospital, a tertiary regional referral centre for patients with advanced HF. Inclusion criteria were reduced LVEF (LVEF <40%), no HF hospital admission in the 2 months prior to enrolment. Patients with cancer within 5 years of enrolment or active inflammatory conditions were excluded as these diseases may affect CPC levels.

Patients were followed for a maximum of 2 years with clinical visits every 6 months. CPC levels were measured at each visit and in 126 patients, peak VO_2 was concomitantly measured. No measurements were done after patients underwent heart transplantation (5 patients) or mechanical circulatory support implantation (9 patients). The institutional review board approved this study and informed consent was obtained from the study patients.

Blood sampling and peripheral blood mononuclear cell isolation

Venous blood samples (21 millilitres) were collected at each clinic visit using BD Vacutainer CPT tubes (Becton Dickinson, San Jose, CA). This sample was stored at room temperature and processed within 2 hours of collection. We isolated peripheral blood mononuclear cells (PBMC) by Ficoll-density gradient centrifugation. We washed recovered cells twice with phosphate buffered saline (PBS) with 2% fetal bovine serum. We re-suspended the cells in CFU-Hill Liquid Medium (StemCell Technologies), then used them to measure CPCs and cultured EO-CFUs.

Early-outgrowth colony-forming units

As previously described [15], batches of 5 million re-suspended cells were placed on fibronectin coated 6-well plates (Biocoat, Becton Dickinson Labware) for 24 to 48 hours to remove contaminating mature endothelial cells and platelets. One million non-adherent cells were then re-plated on fibronectin-coated-24-well plates (Biocoat, Becton Dickinson Labware) using 4 wells per patient. We counted colonies 3 days later. A colony was defined as a central core of round cells with radiating elongated spindle-like cells at the periphery. One colony represented one EO-CFU. We expressed values as mean EO-CFU per well. The inter-rater reliability of this assay was 0.88 [15].

Circulating pro-angiogenic progenitor cells

Circulating pro-angiogenic progenitor cells were identified from isolated PBMC and counted through triple-staining with monoclonal antibodies: Fluoresceinisothiocyanate (FITC)-conjugated anti-CD34 (Miltenyi Biotec, Auburn, CA), R-phycoerythrin-conjugated anti-VEGFR2 (Myltenyi Biotec, Auburn, CA), and

allophicocyanin-conjugated anti-CD133/1 (Myltenyi Biotec, Auburn, CA). This protocol is described in detail in Supplemental Methods. Results were calculated as percentages of CD34+ cells that co-expressed CD133 and/or VEGFR2 antigens on their surface, leading to 3 different cell populations: CD34+CD133+ cells, CD34+VEGFR2+ cells and a sub-population of CD34+CD133+VEGFR2 cells. Cell number was expressed for each patient as number of cells/million PBMC. An investigator blinded to patients' characteristics performed cell counting. Previously described intra-assay reliability was 0.94 [15].

Peak oxygen consumption

Patients underwent cardiopulmonary exercise stress testing (CPET) at each clinic visit. CPET was performed by an experienced technician using a cycle ergometer (Lode MedGraphics, Minneapolis, MN) and metabolic cart (MedGraphics CardiO_2-Ultima, Minneapolis, MN). The work rate was increased using a continuous ramp protocol of 10 watts/minute in all patients. Breath by breath analysis of expired gases was performed. Patients were encouraged to exercise to a respiratory exchange ratio (RER) value $\geq$1.1. The average of the VO_2 levels obtained during the mid 5 breaths of the last 7 breaths was used as a measure of peak VO_2 to eliminate oscillations due to irregular breathing.

Other variables

Clinical and laboratory data, including demographic characteristics (age, sex, race), co-morbidities (diabetes, hypertension, dyslipidemia, peripheral vascular disease, chronic lung disease), HF history (underlying cause, last HF hospital admission, medications) and physical examination (body mass index (BMI), current NYHA class, heart rate and blood pressure at rest) were collected. Laboratory values included

haemoglobin, leucocytes, lymphocytes, electrolytes, BUN (blood urea nitrogen), serum creatinine, total cholesterol, uric acid, BNP (b-type natriuretic peptide) and C-reactive protein (CRP).

Clinical Outcomes

We recorded hospital admissions due to decompensated HF, deaths, HTx and VAD implantation that occurred during follow up. Urgent HTx was defined as listing status 3-4 based on the Canadian listing status at the time of transplant; urgent VAD was defined by an INTERMACS level 1-3 at the time of implant.

Statistical analysis

A detailed statistical analysis is described in Supplementary Methods. We used multivariable mixed-effect models to analyze whether there was an independent association between changes in CPC levels and peak VO_2 and to explore the association between serial measurements of CPCs and EO-CFU We used separate models for CPC levels and EO-CFUs. We determined confidence intervals of estimated coefficients and statistical significance using bootstrapping with 1000 replications.

We evaluated the association between CPC levels and all-cause mortality, first hospital admission due to HF and a composite outcome of deaths, urgent HTx and urgent VAD. We used univariable Cox proportional hazards model for these analyses.

RESULTS

Table S1 in Supplementary material presents demographic characteristics for the 126 patients. The mean age was 55 (SD 11 years, minimum 24 and maximum 78 years)

and 97 (77%) patients were male. Overall, patients were on optimal medical therapy (96% were using β-blockers, 94% inhibitors of the renin-angiotensin system and 57% had an internal cardiac defibrillator). Seventy-eight percent of patients were NYHA class II to IV and 43 (34%) patients had ischemic cardiomyopathy.

Association between circulating progenitor cells and peak oxygen consumption

During a median follow up of 7 months (inter-quartile range of 4 months), there were 270 concomitant measurements of CPC levels and peak VO_2; the number of repeated measurements on each patient varied from 1 to 4; 91 (72%) patients had 2 measurements, 48 (38%) had 3 measurements and 5 (4%) patients had 4 measurements.

The median value of EO-CFU was 11 units/well (IQR of 23 units/well). The median value of CPCs at the first measurement was 10 cells/million PBMC (IQR of 20 cells/million PBMC) for CD34+VEGFR2+ cells, 175 cells/million PBMC (IQR of 220 cells/million PBMC) for CD34+CD133+ cells and 1.5 cells/million PBMC (IQR of 3 cells/million PBMC) for CD34+VEGFR2+CD133+ cells. There were 15 (10%) patients with undetectable levels of CD34+VEGFR2+CD133+ cells throughout the follow up. Baseline mean peak VO_2 was 14.6 with SD of 4 mL/kg/min.

Changes in EO-CFU and CD34+VEGFR2+ cells were independently associated with changes in peak VO_2 as shown in Table 1 and 2. We found a significant interaction effect between EO-CFU and diabetes (p=0.02). Based on the results from the multivariable mixed-effect analysis, in non-diabetic patients, an increase of 10 units/well in EO-CFU was independently associated with an increase of 0.27 mL/kg/min in peak VO_2 (p=0.01) (Figure 1).

In patients with diabetes, decreased levels of EO-CFU were associated with better functional capacity. A decrease of 10 units/well in EO-CFU was independently associated with an increase of 0.2 mL/kg/min in peak VO_2 (p=0.02) (Figure 1). There were no statistically significant effects of other cardiovascular risk factors on the association between EO-CFU and peak VO_2.

CD34+CD133+ cells and CD34+VEGFR2+CD133+ cells were not significantly associated with peak VO_2. However, an increase in 10 CD34+VEGFR2+ cells/million PBMC was independently associated with an average decrease of 0.2 mL/kg/min in peak VO_2 ($p<0.05$). The investigation of interaction effects between CD34+VEGFR2+ cells and cardiovascular risk factors suggested that this association was not significantly modified by the presence of increased age, male sex, ischemic cardiomyopathy or obesity. In patients with diabetes, lower CD34+VEGFR2+ cells were associated with decreased peak VO_2 (Figure 2); however, this effect lost statistical significance after validating our results by bootstrapping (Table 2).

Other factors significantly associated with decreased peak VO_2 were older age, female sex, ischemic cardiomyopathy, higher BMI and poorer NYHA class.

Association between circulating progenitor cells and colony forming units

Based on the multivariable mixed-effect analysis, higher levels of CD34+VEGFR2+ cells were independently associated with lower EO-CFUs adjusted for patient functional capacity as measured by peak VO_2 and diabetes (Figure 3). In patients with similar peak VO_2, an increase of 100 CD34+VEGFR2+ cells/million PBMC was significantly associated with a decrease of 12 EO-CFUs/well (p=0.02). This association

was not significantly different in non-diabetic or diabetic patients (p value of the interaction = 0.88). There was no significant association between EO-CFU and CD34+CD133+ cells or CD34+VEGFR2+CD133+ cells.

Association between circulating progenitor cells and outcomes

During a median follow up of 7 months (inter-quartile range of 4 months), we collected 444 CPC measurements in 156 patients (139 (89%) patients had 2 measurements, 188 (76%) had 3 measurements and 31 (20%) patients had 4 measurements). Twelve patients died (all due to cardiovascular reasons), 5 patients underwent urgent VAD and 3 patients received an urgent HTx; 44 patients were admitted to the hospital due to decompensated HF.

Table 3 shows the analysis of the association between CPC levels and outcomes. A 10-unit increase in EO-CFU was associated with a significant 4-fold reduced mortality risk (HR 0.25, 95%CI 0.0.9-0.69) and a 1.5-fold reduced risk for the composite outcome (HR 0.67, 95%CI 0.45-0.98). EO-CFUs were not associated with hospital admissions. Levels of CD34+VEGFR2+ cells were not associated with an increased risk of mortality, composite outcomes or hospital admissions.

DISCUSSION

In this study on ambulatory heart failure patients we found that a decrease in CD34+VEGFR2+ cells and an increase in EO-CFUs were associated with increased functional capacity as measured by peak VO_2. Increased EO-CFUs were associated with a reduced risk of death and of a composite outcome of death, urgent HTx and VAD. In

patients with diabetes, higher EO-CFUs were associated with poorer functional capacity. Circulating progenitor cells, identified through the expression of antigens (CD34+, VEFGR2+ and/or CD133+ cells) or culture (EO-CFU), mainly represent hematopoietic progenitor cells, which may participate in adult neo-vasculogenesis by promoting vessel formation and repair through a paracrine mechanism [13].

Circulating pro-angiogenic progenitor cells in non-diabetic patients

Circulating progenitor cells consist of a heterogeneous group of cells expressing CD34, VEGFR2 and/or CD133 antigens that are mobilized in response to endothelial damage after myocardial infarction [16], bypass surgery [17], active Kawasaki disease [18] or burn injury [19]. Increased numbers of CPCs have been associated with disease severity. During the course of an acute myocardial infarction, higher levels of CPCs were associated with more profound clinical hemodynamic decompensation and higher mortality [20].

EO-CFUs provide a way to assess CPC function and the potential for cellular proliferation. Patients with lower levels of EO-CFUs have reduced angiogenic potential, which could contribute to the development of more prominent atherosclerosis and increased disease severity. Lower EO-CFUs are associated with a higher burden of subclinical atherosclerosis in the coronary arteries, aorta and carotid arteries [21,22]. In addition, in patients with documented coronary artery disease, low levels of EO-CFUs are independently associated with increased disease severity [23] and progression [24].

In our study, we found that increased EO-CFUs were associated with reduced mortality. To our knowledge only one study [25] has previously explored the association

between EO-CFUs and mortality in HF patients. In contrast to our results, Michowitz et al found that increased EO-CFU were independently associated with higher mortality and impaired NYHA class [25]. The difference in results may be explained in part by model overfitting, which may lead to untrustworthy associations by chance. Michowitz et al included 13 predictors in their multivariable model, a high number in comparison to the small number of outcomes reported (21 deaths).

Our study showed an inverse association between circulating CD34+VEGFR2+ cells and functional capacity as a measure of HF severity. We hypothesize that greater CD34+VEGFR2+ cell mobilization may be a consequence of more pronounced endothelial injury and dysfunction. In addition, lower EO-CFUs may represent decreased vascular repair potential and subsequent impaired vascular function. The involvement of these cells in the process of vasculogenesis, repairing lost and dysfunctional endothelium, may be an integral component to maintain cardiovascular health.

Endothelial dysfunction is important in patients with HF reducing myocardial perfusion and promoting ventricular dysfunction as a consequence of the reduced endothelial-dependent vasodilator capacity of coronary and periphery arteries. The imbalanced NO bioavailability and increase oxidative stress also cause direct abnormal changes in the myocardium leading to ventricular dysfunction [9]. Peripheral vasoconstriction causes higher systemic vascular resistance and increased after-load. Elevated pre-load and after-load further augment cardiac workload and worsen symptoms. The poor exercise capacity seen in HF patients may be further aggravated by local vascular dysfunction within skeletal muscle [26].

Based on our results, we believe that vascular function may be the link between functional capacity and CPC levels in HF patients. A beneficial association between endothelial function and CPC function in disease free patients [27] and HF patients has been previously reported [28]. Vascular function plays a central role in the development and progression of HF and may prove an important therapeutic target for future treatment strategies [9].

Circulating pro-angiogenic progenitor cells in diabetic patients

In this study, we observed that the association between CPCs and functional capacity differed in patients with and without diabetes. In diabetic patients, higher EO-CFUs, rather than lower levels, were associated with poorer functional capacity. Furthermore we identified a trend toward decreased CD34+VEGFR2+ cells in diabetic patients with reduced functional capacity.

Previous studies have identified that levels of circulating CPCs and CPC function vary according to the extent and type of vasculopathy in patients with diabetes. Circulating CPCs are lower in diabetic patients with macro-vasculopathy and these levels may significantly increase in patients with micro-vasculopathy, such as proliferative retinopathy [29]. CPC function measured as EO-CFU may be increased in patients with proliferative retinopathy and decreased in diabetic patients with peripheral vascular disease [30].

Diabetic patients may have impaired CPC mobilization and function because of detrimental cell signalling and complex endothelial dysfunction [31]. Co-existing types of macro- and micro-vasculopathy may be a consequence of the so-called diabetic paradox,

with simultaneous reduced vessel formation in ischemic macro-vascular compartments and increased neovascularization in micro-vascular beds [30]. Patients with diabetes often have aggressive multifocal atherosclerosis. This may occur as a consequence of widespread endothelial dysfunction secondary to increased reactive oxygen species (ROS) [32]. Even though our study has limited capacity to explore the pathophysiology associated with this altered behaviour in HF patients with and without diabetes, the different relationship between CPCs and functional capacity in our population may depend on the type and extension of micro- and macro-vasculopathy in diabetic patients.

Association between circulating CPC and CPC function

We observed a significant inverse association between CD34+VEGFR2+ cells and EO-CFUs, likely reflecting a compensatory mechanism involved in the process of adult vasculogenesis. Higher CD34+VEGFR2+ cell levels, released by damaged endothelium, may try to compensate for the decrease in CPC ability to proliferate and repair injured areas.

Study limitations

Due to the small sample size and limited number of events, we were not able to explore if the association between CPC levels and outcome differs in patients with and without diabetes. Studies with more events may elucidate if diabetes modifies this relationship.

Our study provides strong evidence regarding the association between CPCs and HF severity due to the longitudinal study design, which reduces the potential risk of

spurious association of a single measurement characteristic of a cross-sectional study design.

We proposed that the link between CPC levels and functional capacity may be dysfunctional endothelium. However, we did not directly measure endothelial function in our population. We validated our findings using bootstrapping; however, to test their reproducibility, the results described in this study should be replicated in an external cohort.

During follow up, there were no changes in the type of medications prescribed; however, there were non-analyzable changes in medication doses on some occasions based on the attending physician judgement. Diabetic control has an impact on the level of EO-CFUs [34]; in this study, we did not measure glucose control in diabetic patients during follow up. We believe that changes in medications and differences in diabetic control may have introduced some random error by potentially impacting patient functional capacity and/or CPC levels and attenuating their association.

Due to limited resources, we were not able to explore other functional characteristics of CPCs, such as migration, apoptosis, tubular formation, cytokine release and ROS profile. These tests may provide further understanding about the role of CPCs in the pathogenesis of HF.

CONCLUSIONS

The present study demonstrates differential relations between CPCs and outcome in diabetic versus non-diabetic HF patients. Increased EO-CFU and decreased

CD34+VEGFR2+ cells are significantly associated with improvements in functional capacity in non-diabetic HF patients. Higher EO-CFUs were also associated with reduced mortality. Higher CD34+VEGFR2+ cells and decreased EO-CFU are associated with vascular damage and more profound endothelial dysfunction. These findings suggest that improved vascular angiogenic capacity could be associated with better functional capacity and improved survival in HF patients. This association was reversed in patients with diabetes, which may reflect different CPC behaviour depending on the type and extension of micro- and macro-vasculopathy in diabetic patients. The basis for these differences and their significance require further examination in future studies.

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TABLES

Table 1: Mixed effect model estimates analyzing the association between changes in early-outgrowth colony forming units (EO-CFU) and peak VO_2

Variable	**Observed regression coefficient**	**Bootstrap estimates**		
		Mean regression coefficient	**95% CI**	**p**
Age (per 10-year increase)	-0.47	-0.54	-0.8 – -0.07	0.02
Female sex	-2.8	-3	-4 – -1.6	<0.001
Diabetes	-0.95	-1.04	-2.2 – -0.32	0.13
Ischemic cardiomyopathy	-1.56	-1.85	-2.6 – -0.50	0.003
BMI (per kg/m^2)	-0.16	-0.19	-0.25 – -0.08	<0.001
NYHA class	-1.07	-0.77	-1.8 – -0.35	0.003
LV ejection fraction (per 10-% increase)	0.47	0.41	0 – 0.9	0.06
Statins	0.58	0.99	-1.2 – 2.4	0.78
Spironolactone	0.18	0.11	-1.2 – 1.6	0.53
Cardiac resynchronization therapy	-0.90	-0.66	-2 – 0.25	0.13
Creatinine (per 100-µmol/L increase)	-0.64	-0.69	-2.2 – 1	0.43
Haemoglobin (per 10-g/L increase)	0.16	0.19	-0.2 – 0.5	0.40
Lymphocytes (per 10-% increase)	0.23	0.31	-0.3 – 0.8	0.43
Cholesterol (per 1-mmol/L increase)	0.24	0.44	-0.44 – 0.8	0.35
BNP (per 1-log unit increase)	-0.43	-0.38	-0.9 – 0.14	0.06
CRP(per 1-log unit increase)	-0.11	-0.15	-0.8 – 0.6	0.78
EO-CFU (per 10 units/well)	0.28	0.27	0.07 – 0.5	0.01
EO-CFU * Diabetes	-0.54	-0.47	-1 – -0.1	0.02

BMI, body mass index; BNP, b-type natriuretic peptide; CRP, c-reactive protein; EO-CFU; early outgrowth colony-forming units; LV, left ventricular; NYHA, New York Heart Association.

Table 2: Mixed-effect model estimates analyzing the association between changes in CD34+VEGFR2+ cells and peak VO_2.

Variable	**Observed regression coefficient**	**Bootstrap estimates**		
		Mean regression coefficient	**95% CI**	**p**
Age (per 10-year increase)	-0.47	-0.54	-0.8 – -0.01	0.02
Female sex	-2.8	-2.9	-4 – -1.5	<0.001
Diabetes	-2.7	-2.5	-4.1 – -1.2	<0.001
Ischemic cardiomyopathy	-1.8	-2	-2.7 – -0.9	<0.001
BMI (per 1-kg/m^2 increase)	-0.16	-0.18	-0.24 – -0.07	<0.001
NYHA class	-0.97	-0.69	-1.7 – -0.24	0.009
LV ejection fraction (per 10-% increase)	0.45	0.39	-0.04 – 1	0.07
Statins	0.80	1.25	-0.7 – 2.3	0.29
Spironolactone	0.08	-0.04	-1.1 – 1.3	0.89
Cardiac resynchronization therapy	-0.91	-0.57	-2.2 – 0.4	0.17
Creatinine (per 100-µmol/L increase)	-0.68	-0.71	-2.5 – 1.1	0.46
Haemoglobin (per 10-g/L increase)	0.17	0.22	-0.2 – 0.6	0.38
Lymphocytes (per 10-% increase)	0.19	0.25	-0.3 – 0.7	0.48
Cholesterol (per 1-mmol/L increase)	0.24	0.44	-0.32 – 0.8	0.37
BNP (per 1-log unit increase)	-0.39	-0.34	-0.8 – 0.05	0.08
CRP (per 1-log unit increase)	-0.09	-0.02	-0.8 – 0.7	0.82
CD34+VEGFR2+(per 10cells/1^6PBMC)	-0.17	-0.19	-0.34 – -0.003	0.04
CD34+VEGFR2+cells * Diabetes	0.35	0.28	-0.3 – 1	0.29

BMI, body mass index; BNP, b-type natriuretic peptide; CRP, c-reactive protein; LV, left ventricular; NYHA, New York Heart Association; PBMC, peripheral blood mononuclear cells.

Table 3: Cox proportional models analyzing the association between changes in early-outgrowth colony forming units (EO-CFU) and CD34+VEGFR2+ cells and mortality (12 events), the composite outcomes of death, urgent heart transplantation and urgent VAD (20 events) and hospital admission due to decompensated heart failure (HF) (44 events).

Outcome	Hazard ratio	95%CI	p
EO-CFU (per 10-unit increase)			
Mortality	0.25	0.09 – 0.69	0.007
Composite outcome	0.66	0.45 – 0.98	0.04
HF hospital admissions*	0.95	0.80 – 1.13	0.59
CD43+VEGFR2+ (per 10-unit increase)			
Mortality	1.09	0.95 – 1.26	0.20
Composite outcome	1.08	0.94 – 1.24	0.24
HF hospital admissions*	0.93	0.77 – 1.13	0.50

* These models were adjusted for age, sex and NYHA class. The rest of the models were univariable including just CPC levels as a time-dependent covariate.

FIGURE LEGENDS

Figure 1. Association between early-outgrowth colony forming units (EO-CFU) and mixed-effect model adjusted peak oxygen consumption (peak VO_2) in patients with (β=0.26) and without diabetes (β=0.28, p=0.01). This association was significantly different in patients with and without diabetes (p for interaction effect=0.02).

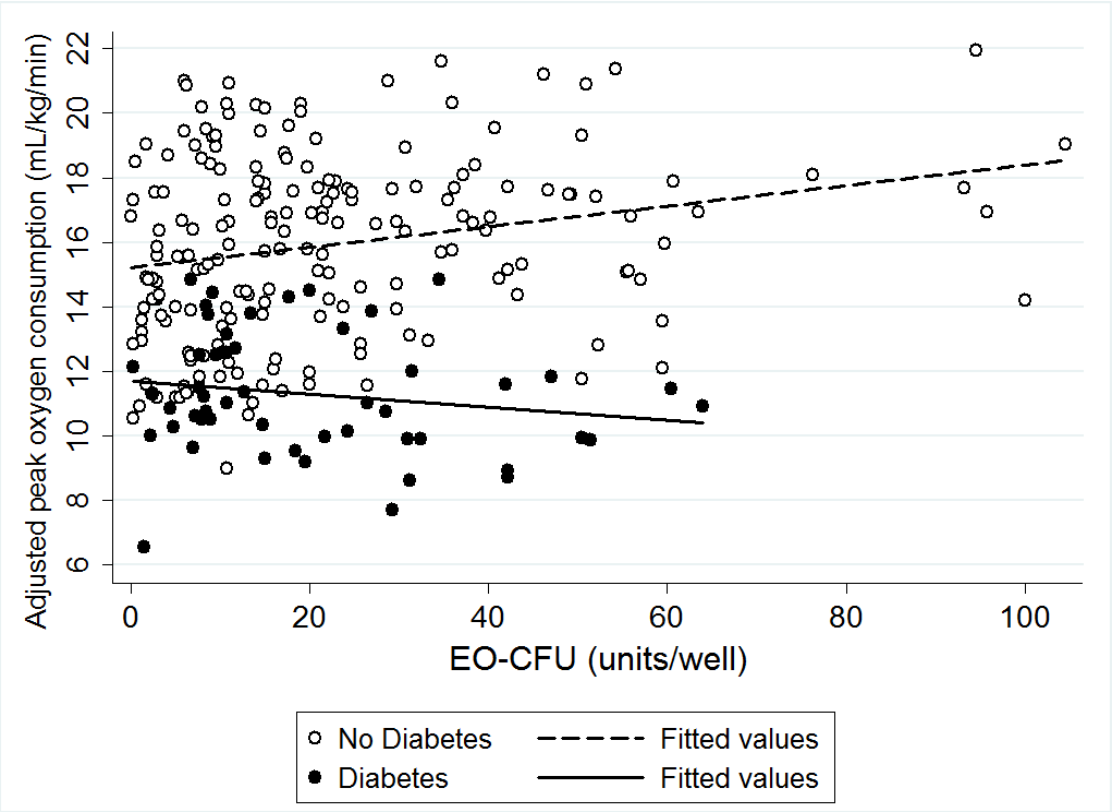


Figure 2. Association between CD34+VEGFR2+ cells and mixed-effect model adjusted peak oxygen consumption (peak VO_2) in patients with (β=0.18) and without diabetes (β= -0.17, p=0.04). This association was not significantly different in patients with and without diabetes (p for interaction effect=0.34).

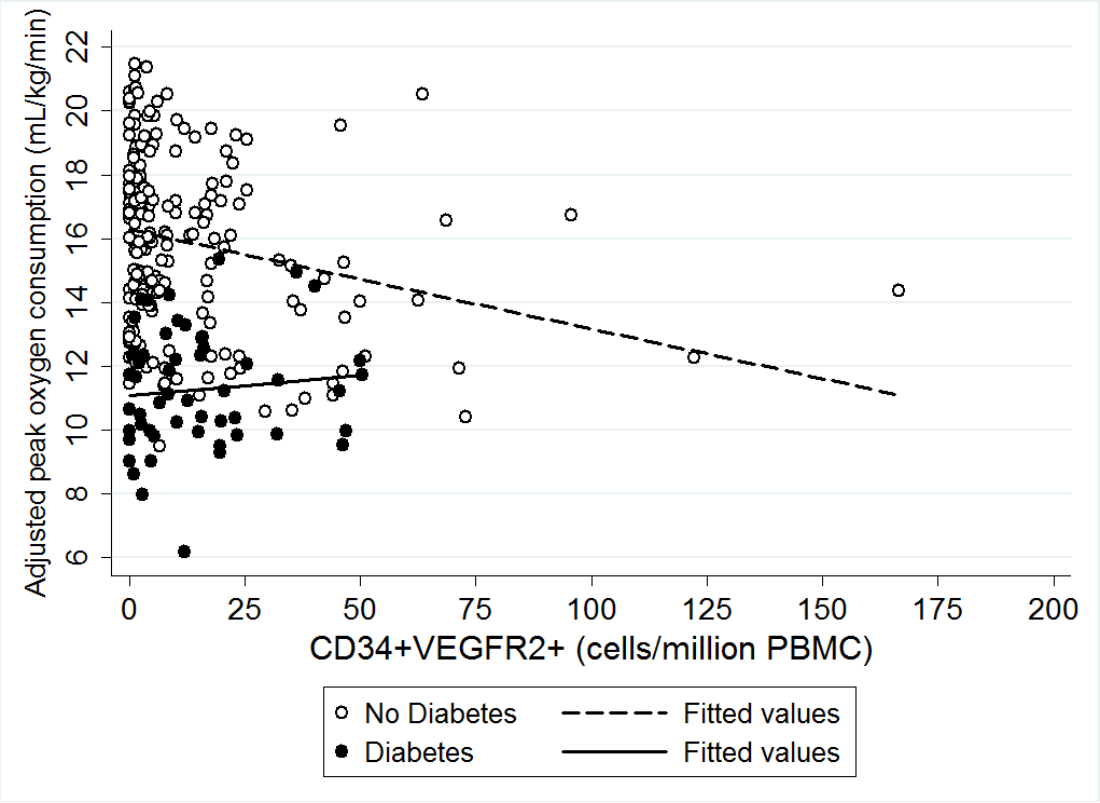
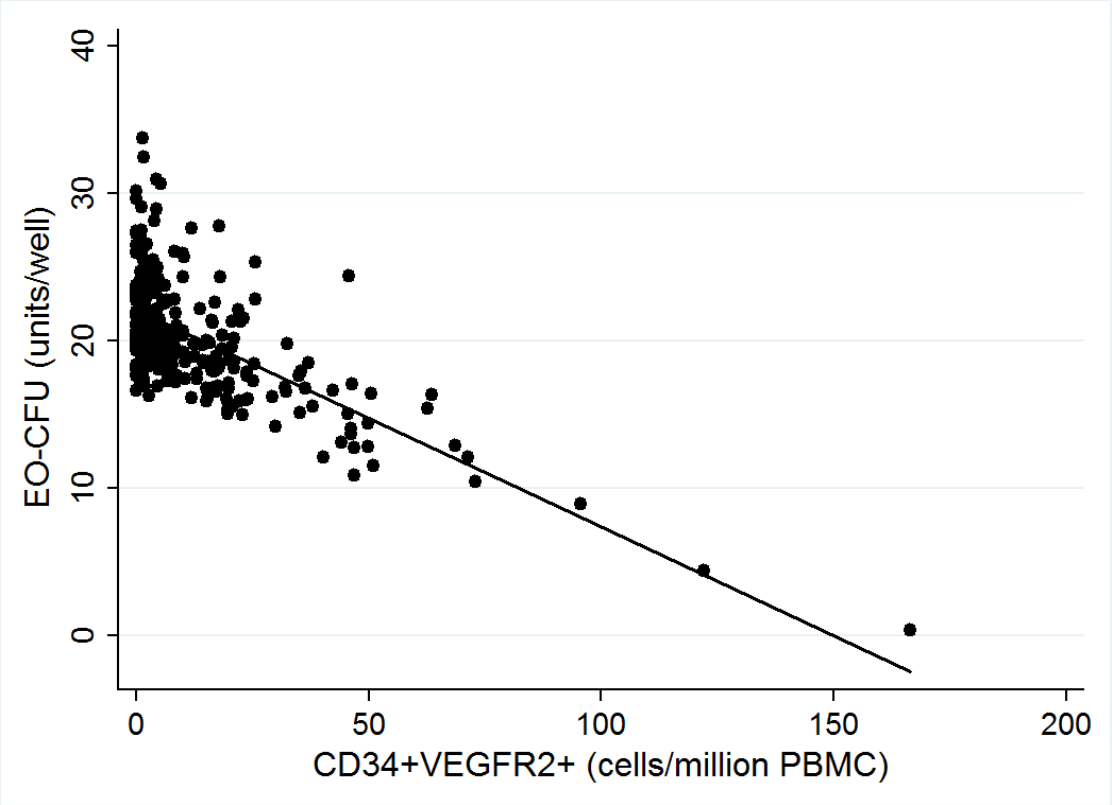


Figure 3. Association between early-outgrowth colony forming units (EO-CFU) and CD34+VEGFR2+ cells mixed-effect model adjusted for peak oxygen consumption and diabetes (β=0.12, p=0.02).



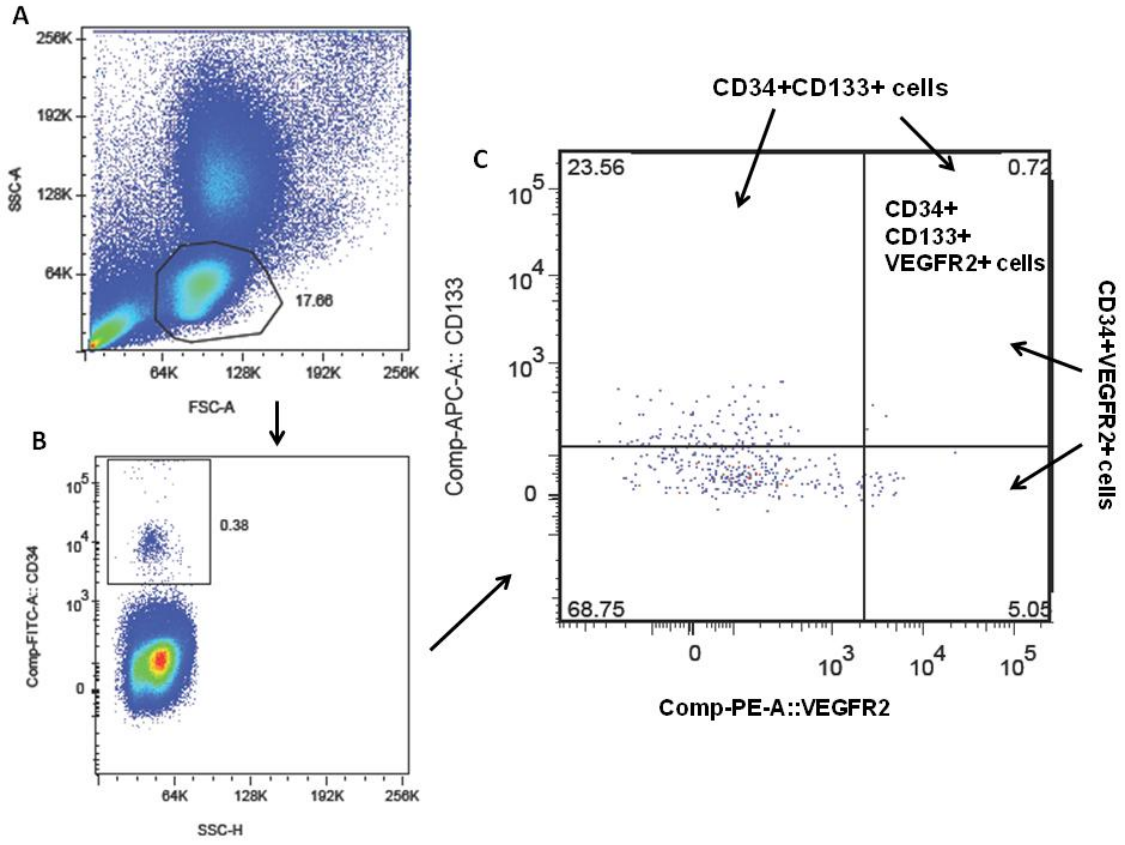
SUPPLEMENTARY MATERIAL

Supplementary Methods S1: Measure of circulating pro-angiogenic progenitor cells by flow cytometry

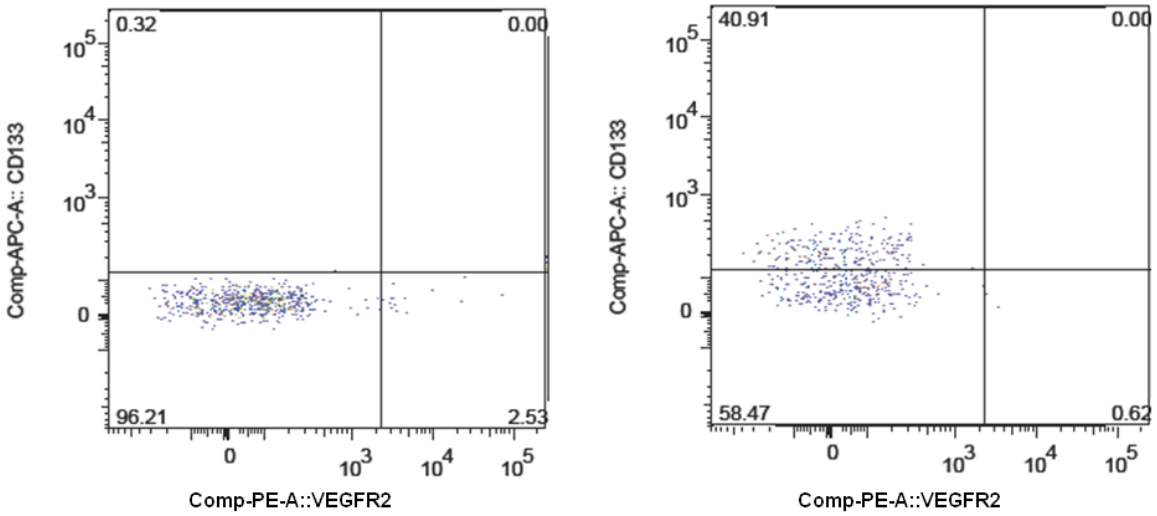
Circulating pro-angiogenic progenitor cells were identified and counted through triple-staining with monoclonal antibodies: Fluoresceinisothiocyanate (FITC)-conjugated anti-CD34 (Miltenyi Biotec, Auburn, CA), R-phycoerythrin-conjugated anti-VEGFR2 (Myltenyi Biotec, Auburn, CA), and allophicocyanin-conjugated anti-CD133/1 (Myltenyi Biotec, Auburn, CA). After cells were re-suspended in CFU-Hill Liquid Medium (StemCell Technologies), 2 million cells were washed with flow buffer, containing PBS, 0.5% bovine serum albumin (BSA) and 2 mM EDTA, then re-suspended and treated for 15 minutes with a FcR blocking agent (Myltenyi Biotec, Auburn, CA) to decrease unspecific binding and finally treated with the antibodies for 25 minutes. Isotype-identical antibodies against human cells (Myltenyi Biotec, Auburn, CA) and samples with combinations of just 2 of the mentioned antibodies served as controls. We processed the samples using the LSR II Flow Cytometer System (Becton Dickinson, San Jose, CA), and analyzed them using FACS Diva software (Becton Dickinson, San Jose, CA). We determined 3 different cell sub-populations with the phenotypes CD34+VEGFR2+, CD34+CD133+ and CD34+VEGFR2+CD133+. We collected a minimum of 500000 events per sample. After excluding cellular debris in a forward scatter/side scatter plot according to their size and granularity and gating on CD34+ cells (panel B), cells were further characterized as CD34+ cells co-expressing CD133 and/or VEGFR2 antigens

(panel C). The immunofluorescence cutoff was set based on controls and kept constant in all scatter plots.

Figure picturing the protocol used to measure circulating progenitor cells by triple staining with CD34, VEGFR2 and CD133 antibodies. Median (and inter-quartile range) proportion of stained cells was 0.23% (0.18) for CD34+cells, 36.5% (24) of CD34+ cells for CD34+CD133+cells and 0.90% (2.4) of CD34+ cells for CD34+VEGFR2+cells. In patients with detectable levels of CD34+CD133+ VEGFR2+cells, the median (and inter-quartile range) was 0.40% (0.52) of CD34+ cells.



The immunofluorescence cutoff was set based on controls and kept constant in all scatter plots. Sample with combinations of just 2 of the mentioned antibodies served as controls (CD34 and VEGFR2 in left panel and CD34 and CD133 in right panel)



Supplementary Methods S2: Detailed statistical analysis

Categorical variables were represented as proportions and continuous variables were summarised by their means and standard deviations (SD) or medians and inter-quartile ranges (IQR). We used mixed-effect models to analyze whether there was an independent association between changes in CPC levels and peak VO_2. Mixed-effect models account for correlations among repeated measurements from the same patient and unequal number of observations per patient. We used random intercepts to allow for heterogeneity between individuals. We selected the covariates based on statistical significance ($p<0.05$) in univariable analyses. Fifteen variables (age, sex, BMI, diabetes, ischemic cardiomyopathy (CMP), LVEF, cardiac resynchronization therapy, statins, spironolactone, NYHA, creatinine, haemoglobin, lymphocytes, BNP and CRP) associated with peak VO_2 at the time of first observation were entered as fixed factors in a multivariable mixed-effect model along with CPC levels and the interactions between CPC levels and age, male sex, diabetes, BMI and ischemic CMP. We subsequently eliminated the non-significant interactions using stepwise backward elimination, in which the least significant interactions were sequentially removed according to a pre-specified p-value of ≥ 0.1. We assessed goodness-of-fit of the model at different stages of model development using a likelihood ratio test. We used separate models for CPC levels and EO-CFUs. We determined confidence intervals of estimated coefficients and statistical significance using bootstrapping with 1000 replications. Estimated regression coefficients express the mean differences of peak VO_2 between groups for binary predictor variables

and the mean change of peak VO_2 associated with one-unit change of the predictor for continuous predictor variables.

We explored the association between serial measurement of CPCs and EO-CFU using mixed-effect models with random intercepts entering peak VO_2 and diabetes as covariates. We also determined confidence intervals of estimated coefficients using bootstrapping with 1000 replications.

Finally, we evaluated the association between CPC levels and all-cause mortality, first hospital admission due to HF and a composite outcome of deaths, urgent HTx and urgent VAD. We used univariable Cox proportional hazards model for the analysis of mortality and the composite outcome; and multivariable Cox proportional hazards model for the analysis of first hospital admission; pre-specified covariates used were age, sex and NYHA class. We entered CPC levels as a time-dependent covariate in all of these models. In the analysis of mortality, patients undergoing VAD and HTx were censored as alive at the time of surgery. We evaluated proportional-hazards assumption by the analysis of the Schoenfeld residuals; results satisfied the assumptions.

SUPLEMENTARY TABLES

Supplementary Table S1: Baseline characteristics of the 156 patients included in this analysis and 126 patients who were able to perform a cardiopulmonary exercise stress testing (CPET).

Variable	**All patients (n= 156)** mean ± SD / n (%)	**Patients with CPET (n= 126)** mean ± SD / n (%)
Age (years)	55 ± 15	55 ± 11
Female sex	31 (20)	29 (23)
Hypertension	65 (42)	54 (43)
Diabetes	37 (24)	27 (21)
Insulin requirement *	18 (48)	10 (37)
Dyslipidemia	92 (59)	74 (59)
Peripheral vascular disease	11 (7)	7 (6)
Body mass index (kg/m^2)	28 ± 6	29 ± 6
Cause of cardiomyopathy		
Ischemic	57 (36)	43 (34)
Idiopathic	74 (48)	74 (59)
Other ‡	25 (16)	9 (7)
β-blockers	148 (95)	121 (96)
ACE inhibitors or ARB	144 (92)	118 (94)
Spironolactone	70 (45)	53 (42)
Statins	85 (54)	74 (59)
Digoxin	55 (35)	40 (33)
Furosemide	111 (70)	85 (67)
Furosemide (mg/day) †	90 ± 65	90 ± 60
ICD	87 (56)	70 (57)
CRT	47 (30)	34 (27)

Supplementary Table S1. Continued.

Variable	**All patients (n= 156)** mean ± SD / n (%)	**Patients with CPET (n= 126)** mean ± SD / n (%)
NYHA class I/II/III/IV	30(19)/62(40)/53(34)/11(7)	28(22)/53(42)/41(33)/4(3)
Peak VO_2 (ml/min/kg)	-	14.6 ± 4
Systolic blood pressure at rest (mmHg)	104 ± 15	104 ± 14
Heart rate at rest (bpm)	70 ± 11	69 ± 11
Left ventricular ejection fraction (%)	30 ± 10	30 ± 8
QRS (msec)	138 ± 40	138 ± 41
Haemoglobin (g/L)	140 ± 16	140 ± 15
Creatinine (μmol/L)	109 ± 47	106 ± 44
Lymphocytes (%)	25 ± 9	25 ± 9
BNP (Median, IQR) (pg/ml)	177, 370	178, 395
Cholesterol (mmol/L)	4.4 ± 1.2	4.6 ± 1.2
C-reactive protein (Median, IQR) (mg/dl)	2, 3	2, 3

* Including just patients with diabetes

‡ Other causes of cardiomyopathy include chemo-induced, peri-partum, congenital and valvular cardiomyopathy.

† Including just patients who were on Furosemide.

ACEI, angiotensin-converter enzyme; ARB, angiotensin II receptor blockers; BNP, b-type natriuretic peptide; BUN, blood urea nitrogen; CRT, cardiac resynchronization therapy; ICD, implantable cardiac defibrillator; IQR, inter-quartile range; NYHA, New York Heart Association; VO_2, oxygen consumption.

CHAPTER VIII

OVERALL DISCUSSION AND CONCLUSIONS

In this thesis, we have identified many challenges in the accurate assessment of prognosis in heart failure (HF) patients present many challenges. Some of the reasons were related to:

- Limitations in the development and performance of existing predictive models
- Lack of incorporation of important mortality predictors in current HF predictive models
- Limited evidence on potentially important prognostic factors
- The identification of new prognostic factors that may enhance the performance of pre-existing predictive models
- Absence of assessment of model clinical applicability

Limitations in the development and performance of existing predictive models

In a systematic review, we identified 20 event-free survival prediction models in ambulatory HF patients. Seventy-five percent (15 of 20 models) were not validated in external cohorts. The lack of testing a model in a different cohort from the one that it was derived limits its clinical applicability for the following reasons.

First, models tend to have above average discrimination and better calibration when tested in the cohort from where the model was derived. Only 6 of these 15 models were internally validated by bootstrapping; they showed adequate to great discriminatory capacity (c-statistic 0.75 to 0.84). As mentioned, it is expected that their performance will be inferior in subsequent validation cohorts but to what extent remains unknown. The 2 models with the highest discrimination [1,2] (c-statistic of 0.77 and 0.84 respectively) included predictors such as left ventricular dyssynchrony measured by myocardial resonance imaging and exercise parameters including oxygen uptake efficiency slope (OUES) and resting end-tidal carbon dioxide pressure, which limit their clinical application in many centers, thereby limiting the appeal for further exploration of their performance in external cohorts.

A second problem may be related to the presence of true differences between the derivation cohort and the cohort in which the model will be applied. This factor may also affect the performance of already externally validated models.

Of the 5 externally validated models, only two, the Heart Failure Survival Score (HFSS) and the Seattle Heart Failure Model (SHFM), were validated in more than 2 independent cohorts, mostly reporting modest (0.70-0.80) to poor discrimination (<0.70).

Some reasons for limited model performance were that these two models were derived from cohorts of HF patients from the early 1990s. Heart failure management and patients profile have changed substantially over time; hence, a model derived from HF patients from 20 years ago will give inaccurate predictions in contemporary HF patients. This is demonstrated by the fact that for example the HFSS performance showed a decline over time.

Not only may the nature and magnitude of relationship between a predictor and an outcome change over time but also the detection of new important predictors may limit older models.

Lack of incorporation of important mortality predictors in current HF predictive models

One of the most important changes in the management of patients with HF is the increased use of internal cardioverter defibrillator (ICD). Currently, approximately half of symptomatic HF patients are treated with an ICD [3,4]. A recently developed model, the SHOCKED predictors [5], was derived from ICD patients included in the Medicare database. This model has demonstrated adequate performance in a validation cohort (c-statistic of 0.74 with excellent calibration). However, the exclusion of some important mortality predictors may limit the SHOCKED's performance.

In a meta-analysis including 72 studies involving 257,692 ICD patients, we identified independent predictors of overall mortality in ambulatory ICD patients. We found that age, baseline renal function, history of heart failure, chronic obstructive

pulmonary disease, diabetes, peripheral vascular disease, left ventricular ejection fraction, NYHA class, atrial fibrillation, wide QRS and the occurrence of appropriate or inappropriate ICD shocks predicted mortality. We used a customized version of the Grading of Recommendations, Assessment, Development and Evaluation (GRADE) approach and observed that confidence in estimates was reduced mainly due to the presence of inconsistency and indirectness. Inconsistency, or variability in results, was higher when predictors were treated as continuous instead of categorical variables (i.e. in the case of age, left ventricular ejection fraction and renal function). Indirectness, or the presence of differences in populations, predictor or outcome, was related to the use of different or unknown definitions to characterize predictors (i.e. in the case of congestive HF, NYHA class, renal function as binary variable and ischemic cardiomyopathy).

Based on the findings of our meta-analysis, we anticipated that the performance of the SHOCKED predictors in a validation cohort might be compromised by the omission of important mortality predictors in ICD patients, including wide QRS, history of peripheral vascular disease, and ICD shocks during follow up. In addition, 3 of the 7 SHOCKED predictors (age, renal function and left ventricular ejection fraction) are continuous variables treated as categorical; this increases uncertainty in the effect estimate associated with these predictors and limits overall model performance.

Motivated by the limitations associated with the SHOCKED predictors, we built the HF Meta-Score, a predictive model based on the findings of our meta-analysis. In this model, we included the 10 independent mortality predictors that we identified, including 3 continuous variables: age, left ventricular ejection fraction and glomerular filtration

rate, and 7 dichotomous variables: diabetes, chronic obstructive pulmonary disease, peripheral vascular disease, atrial fibrillation, NYHA class III-IV, wide QRS (QRS >120 milliseconds) and ICD shock (appropriate or inappropriate shocks). We compared the performance of the HF Meta-Score in predicting mortality to the SHOCKED predictors and found, as we had anticipated, that the HF Meta-Score has better calibration and discrimination.

Limited evidence on potentially important prognostic factors

One of the limitations of the HF Meta-Score is that it only includes established risk factors. This limitation is inherent to the strategy used to develop the HF Meta-Score, which restricts the solutions to this issue to future studies. New studies appraising the additive predictor value of factors alleged as important may overcome this constraint.

Current guidelines recommend the use of peak oxygen consumption (peak VO_2) to guide the transplant selection process in ambulatory HF patients [6]. It has been proposed that peak VO_2 <12 ml/kg/min may identify HF patients who might benefit from cardiac transplantation. This threshold was proposed by a study in which only 23% of the patients were treated with an ICD [7]. A recent study using univariable analysis suggested that survival in ICD patient treated medically dramatically declines only in patients whose peak VO_2 is <10ml/kg/min, advising a lower cut-off in this group of patients [8]. Current guidelines recommend the use of peak VO_2 in the clinical decision process of the management of advanced HF patients, there is no substantial evidence regarding whether peak VO_2 remains an independent mortality predictor in current HF patients, or the

magnitude of this association. The evaluation of the additional predictive value of peak VO_2 over current HF predictive models remains unexplored and an interesting matter to pursue.

A similar scenario can be described with laboratory prognostic markers. For example, brain natriuretic peptide (BNP) is a strong mortality predictor in patients with acutely decompensated HF. We identified no evidence supporting the use of BNP in ambulatory ICD treated HF patients. Incorporation of these unexplored factors to current HF predictive models may aid physicians in further identifying high risk patients who may benefit from advanced HF therapies and end of life discussions.

The identification of new prognostic factors that may enhance the performance of pre-existing predictive models

We have identified that circulating progenitor cells (CPC) are independently associated with functional capacity in ambulatory HF patients. By univariable analysis, we have also described that CPC are strongly associated with mortality.

Circulating progenitor cells are involved in the process of adult vasculogenesis and endothelial damage and repair. Vascular dysfunction is involved in the pathogenesis of HF. The presence of endothelial dysfunction is associated with poorer outcomes in HF patients. None of the current HF models included predictors associated with endothelial function. Whether the inclusion of these factors is useful remains unexplored.

In our study, we identified that CPC measured as early-outgrowth colony forming units (EO-CFU) were associated with mortality in ambulatory HF patients. While this

association seems strong (HR for a 10 unit-increase 0.25, 95%CI 0.09-0.69), it was described in univariable analysis due to the small sample size. Other studies have identified increased levels of EO-CFU as an independent predictor of mortality and poorer outcomes in patients with coronary artery disease [9], acute lung injury [10] and chronic renal dysfunction [11]. Increased EO-CFUs represent increased potential for vascular repair and subsequent improved vascular function. The involvement of these cells in the process of vasculogenesis in different diseases, repairing lost and dysfunctional endothelium, may be fundamental in maintaining cardiovascular health. Future studies assessing the independent association between EO-CFU and mortality in ambulatory HF patients are necessary.

Results regarding circulating progenitor cells identified by flow cytometry as circulating cells co-expressing different antigens, such CD34, VEGFR2 and CD133, still remain challenging to interpret. The main reasons are related to the lack of consensus to characterize these cells and the lack of specificity of these assays. Our study showed that CD34+VEGFR2 cells were not significantly associated with mortality in ambulatory HF patients. This may be partially explained by the small sample size. Nonetheless, uncertainty regarding the predictive value of circulating progenitor cells identified by flow cytometry remains significant, substantially limiting its clinical utility.

Absence of assessment of model clinical applicability

One of the main utilities of a prediction rule is to assist in the medical decision making process differentiating patients that may benefit from a specific intervention or

test from those patients that may benefit from conservative management. The clinical implications of the use of the models discussed in this thesis have not been evaluated.

Many investigators are satisfied by providing a measure of association (i.e. hazard ratio) and the statistical significance between a score and the outcome. It is difficult to assess a model's clinical applicability based on this information. If sample size is large, an association may be statistically significant but small in magnitude and, if the risk is very low in patients without the particular risk factor, imply only a very small increase in absolute risk.

It is also the case that the use of a relative risk measure may be misleading if one fails to take into consideration the baseline risk. For example, if high and low risk patients have significantly different relative risks based on a score, from which one may infer that high risk patients benefit from an intervention while low risk patients do not. This simple assumption is incorrect: low risk patients may still have high enough baseline risk, and thus a great enough risk difference, to gain an important benefit from a specific intervention.

It has been proposed that a clinically useful model should have adequate discrimination and excellent calibration. In models to predict survival, discrimination can be expressed as the capacity of a model to differentiate patients who will die sooner from those who will die later while calibration relates to how the accurate predicted survival is in comparison to the observed survival. Based on the results from our systematic review, calibration was usually under reported. A model could have great discrimination with

poor calibration or vice versa. Both properties are relevant; therefore, both should be reported.

The separate report of discrimination and calibration may confuse physicians. For example, it is difficult to decide if a model with better discrimination might be more clinically relevant than a model with better calibration. In order to overcome this problem, new techniques that combine both discrimination and calibration have been proposed. These include reclassification tables, reclassification calibration statistics and net reclassification and integrated discrimination improvements. When comparing two models, these techniques may help to assess which performs better in a specific group of patients or if a new prognostic marker adds clinically relevant information over a pre-existing model. None of the 20 models identified in our systematic review used these techniques. In fact, only two studies compared discrimination between models.

In developing a new model or testing the value of a pre-existing one or a new predictive factor, the use of these techniques provide important information because they offer prognostic information at different risk strata that can better guide clinical decision making. However, this information may be insufficient if it is not evaluated using analytical decision models. Analytical decision models are helpful when considering risks and benefits associated with particular medical decisions, especially when other types of comparative studies are not feasible.

Based on the findings of this thesis, there is still a gap between the development of a good predictive model to assess prognosis in ambulatory HF patients and the clinical implications of its use. It appears that the HF Meta-Score is a promising score because it

includes important mortality predictors in current HF patients and it has shown adequate performance as assessed by reclassification tables. However, further work is required to address if other predictors, including but not limited to peak VO2, BNP or EO-CFU, have additional prognostic information. The clinical utility of the HF Meta-Score and the incorporation of any other predictive factor should be tested considering risks and benefits through medical decision analysis.

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