Limited added value of circulating inflammatory biomarkers in chronic heart failure

Nymo: Multiple biomarkers in chronic heart failure

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Abstract

OBJECTIVES/BACKGROUND: Modeling combinations of multiple circulating markers could potentially identify heart failure (HF) patients at particularly high risk and aid in the selection of individualized therapy. We evaluated if a panel of biomarkers improved prognostication in patients with HF with reduced ejection fraction of ischemic origin using a systematized approach according to suggested requirements for validation of new biomarkers

METHODS: From a panel of 20 inflammatory and ECM biomarkers two different biomarker panels were created and added to the Seattle heart failure score, and the prognostic model from the CORONA study (n=1497) which included conventional clinical characteristics and C-reactive protein and N-terminal pro B-type Natriuretic Peptide. Interactions with statin treatment were also assessed.

RESULTS: The two models, which were composed of (Model 1) endostatin, interleukin (IL)-8, sST2, TnT, galectin-3 and CCL21 and (Model 2) Troponin T, sST2, galectin-3, pentraxin 3, and sTNFR2 significantly improved the CORONA and Seattle HF models, but added only modestly to their Harrell's C statistic and NRI. In addition, there was no effect of rosuvastatin on the levels of a wide range of inflammatory and ECM markers, but there was a tendency of patients with lower level of biomarkers in the two panels to have a positive effect of statin treatment.

CONCLUSIONS: A multi-marker approach using the particular panel of biomarkers measured, in the specific HF patient population studied, was of limited clinical value for identifying future risk of adverse outcomes.

Abbreviations

CORONA - The Controlled Rosuvastatin Multinational Trial in Heart Failure
CV – cardiovascular
ECM – extracellular matrix
HF – Heart failure
LVEF – left ventricular ejection fraction
NRI – net reclassification index
PS – Prognostic score
SHFS – Seattle heart failure score

Introduction

The prognosis in heart failure (HF) remains poor despite improvements in disease management. Persistent inflammation and extracellular matrix (ECM) remodeling are considered central pathogenic elements in HF progression.(1) As a result of their role in the pathogenesis of HF, circulating inflammatory and ECM markers may also be convenient, noninvasive, tools for risk stratification and prognostication in these patients.(2)

We have previously evaluated a range of biomarkers in The Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA) trial, comprising elderly patients with moderate to severe, ischemic HF.(3-16) Classifying these markers according to categories proposed by Braunwald, revealed a good coverage of the different pathological pathways activated in HF (Figure 1), with a focus on inflammation and matrix remodeling.(2) When assessed separately, several of these markers provided independent prognostic information or identified subgroups of patients who seemed to benefit from rosuvastatin therapy. However, the improvement in prognostic discrimination as evaluated by net reclassification index (NRI) and Harrell's C-statistics (C), beyond established clinical risk factors and in particular N-terminal pro B-type Natriuretic Peptide (NT-proBNP), was relatively modest and their clinical usefulness unclear.

While measurements of individual markers of inflammation and the EMC so far have not improve risk stratification of patients with HF in a clinically meaningful way, combinations of multiple markers might help identify subjects with a clinically significantly increased risk. The combination of multiple markers might also help select patients for individualized therapy. The idea of a multimarker approach has been around for several years, but few studies have tested the power of such models, and most of these trials included few biomarkers or examined small populations. Moreover, the lack of optimal adjustment for existing tests and the lack of internal or external validation may have biased results.(17-19)

In the present study, we used a systematized approach to assess the prognostic value of a combination of biomarkers from the CORONA trial.(20)

Methods

For full description of methods, see supplementary data. A flowchart showing the statistical approach is shown in Figure 2. Briefly, the CORONA population was divided into three sub-groups. Subgroup one had no biomarker data and was used for fitting a Cox model including routine clinical and biochemical variables as previously reported (history of diabetes, LVEF, BMI, NYHA class, ApoB/ApoA-1 ratio, history of intermittent claudication, gender, age, heart rate and eGFR, CRP and NT-proBNP).(21) The Cox model was then used to calculate a prognostic score (PS) by multiplication of estimated coefficients with corresponding variables for each individual subject in the biomarker population. Seattle HF score (SHFS) was calculated based on the available data.(22) As sodium levels, lymphocyte count, as well as hemoglobin levels and uric acid were not available in the CORONA dataset, these where excluded from our SHFS.

Results

Model building

Demographics of the CORONA inflammatory sub-study and the training and validation set are provided in Table S1. No significant differences between the training and validation sets were observed. All previously measured biomarkers in the CORONA database were entered as potential variables for the multimarker approach, i.e. biglycan, mimecan, endostatin, YKL40, galectin 3, IL8, MCP1, CxCL16, CCL21, sST2, troponin T, SFRP3, OPG, NGAL, pentraxin 3, sTNFR1, sTNFR2, IL6, sGP130 and TNF. The three different approaches to building a model from available biomarkers yielded three slightly different results. By keeping all variables as proposed by at least two methods, six variables remained in Model 1 (i.e. endostatin, IL-8, sST2, TnT, galectin-3 and CCL21; Table 1). Testing the variable selection by bootstrapped model selection showed that all biomarkers chosen by an approach were selected in at least 50% of the repetitions, and no other biomarkers were selected by multiple approaches in more than 50% of the repetitions (Table S2). For Model 2, we included more established HF risk markers from the literature (i.e. TnT, sST2, galectin 3, pentraxin 3, and sTNFR2; Table 1). (7,23-27)

Performance of the multimarker models

The prognostic scores (PS) based on only the variables included in Model 1 and Model 2, respectively,

were significantly associated with outcome in the validation set. However, the scores from each model performed worse than the original CORONA PS (table 1). When the combined biomarker scores from each of the two models were added to the CORONA PS, the models showed reasonable calibration by a Groennesby and Borgan test score (Figure 3 and table S4), as well as on visual inspection of Arjas-like plots in tertiles of PS. However, there was a tendency in Model 1 to overestimate events in the low risk group, but both were well calibrated in the other tertiles (Figure 4). Model coefficients for both models are given in Table S3.

The addition of each biomarker model to the CORONA PS provided better results than the CORONA PS alone as judged by a likelihood ratio test, but there was no significant improvement in Harrell's C statistics or Gönen and Heller's K-statistics for any endpoint (Figure 3 and table S4). However, the addition of each biomarker model lead to a small, but significant improvement in NRI for all endpoints, except for cardiovascular (CV) mortality in Model 1 (Figure 3). This was mainly due to patients without event getting a lower risk score (Table S4).

Comparison with the Seattle Heart Failure Score

When we used the SHFS as the base model instead of the original CORONA PS, the addition of either biomarker model markedly improved discrimination for all endpoints (Table S6). When adding NT-proBNP to the SHFS as a base model, this was no longer the case. However, NRI remained significant for all outcomes, and there was a significant change in C-statistics for the primary endpoint in CORONA for both models (Figure 3 and table S5).

Effect of statin treatment on markers of inflammation

In the CORONA trial, patients were randomly assigned to treatment with rosuvastatin or placebo. We were therefore able to investigate if three months of statin treatment influenced biomarker levels in patients with HF. As shown in table S7, the relative change in biomarker levels differed between the treatment arms only for biglycan, YKL40, CXCL16 and PTX3. Biglycan and PTX3 increased more in the rosuvastatin group, while CXCL16 and YKL40 increased more in the placebo group. Since patients with low level of biomarkers may have a limited potential for benefitting from the anti-inflammatory effect of statins, we also assessed treatment effects in the top two tertiles for each marker. In these patients, the result remained similar for PTX3, YKL40 and CXCL16 with a

significant relative change in the same direction as previously (Table S8).

Effect of statin treatment in different risk groups.

Finally, we evaluated the interaction between the PS of Model 1 and 2, and effect of rosuvastatin, treatment on outcome. For all-cause mortality, there was a borderline significant interaction between rosuvastatin treatment and Model 1 PS. Patients in the lowest tertile PS had a significant effect of statin treatment, while this was not the case for any of the other patients. We got similar results when testing interaction for Model 2 PS, suggesting that patients with little inflammatory activity at the baseline of study had some effect of rosuvastatin treatment, compared with those with more inflammation (Figure 5). Similar patterns were found for CV mortality and the primary endpoint, but only Model 2 had a significant interaction with treatment for the primary endpoint.

Discussion

Previous studies have suggested that panels of multiple biomarkers may add prognostic information to established predictive metrics in chronic HF(18,28-30). In this study, we were only partly able to confirm this hypothesis. While two slightly different panels of biomarkers added information to the SHFS and improved NRI, even when NT-proBNP was added to the model, the clinical relevance of these markers are uncertain. Furthermore, when comparing the two models to the previously published CORONA model, there was only a small, but significant NRI. Thus, whereas these data suggest that NT-proBNP is a useful prognostic biomarker in elderly patients with HF of ischemic origin, the added value of inflammatory and ECM related biomarkers seems to be limited. Finally, our study does not support a direct anti-inflammatory effect of statin therapy in elderly patients with ischemic HF, but suggests that patients with a lower inflammatory burden may benefit from statin therapy.

We used two models to test the prognostic potential of a multimarker approach in our patients. Our selection of biomarkers from the literature (i.e. Model 2) was based on the authors' judgement of biomarkers that have repeatedly been suggested, or have been shown to be associated with outcome in several previous studies, and was available in this study. (7,23-27) However, few publications advocating these biomarkers fulfill suggested requirements for validation of new biomarkers.(17) Most studies reporting prognostic abilities of new biomarkers, including our studies in CORONA,

include the marker in regression analysis adjusted for known prognostic variables and scores. However, this approach is known to give overly optimistic estimates of the model's performance.(31) While internal validation is done in a few studies, external validation of suggested biomarkers in new populations is lacking, making it difficult to choose biomarkers likely to perform well in a new population. Our selection of biomarkers in Model 2, however, performed better than a model created by automatic variable selection (Model 1), suggesting that the aggregation of published data may give useful information for selection of candidate biomarkers.

Measures like the NRI and C-statistics may be used for the quantification of the usefulness of a new biomarker, but what may be considered clinically significant changes of these measurements is still an open question.(32) Furthermore, the lack of statistical significance of these measures, and in particular the C-statistics, could be due to limited power of the study. However as suggested by the narrow confidence intervals of the change in C-statistics, our study had enough power to detect very slight changes in the order of 0.02, and we think that smaller changes would give little clinical meaning. In addition, NRI and C-statistics are overly optimistic when applied to the same population as the model is developed in. We compensated for this by internally validating our models. However, even with this, performance may still be worse when applied to a different population with different characteristics.

The choice of implementing a new biomarker in clinical use depends on many factors, among which is cost. If available biomarkers such as CRP and NT-proBNP, give the same prognostic information as new biomarkers, this substantially reduces their usefulness. Thus, candidate biomarkers should provide added information not only on top of established risk scores such as SHFS, but also to available and widely measured prognostic markers such as NT-proBNP and CRP.(33) In our study, while both biomarker panels added significant information to SHFS, the added information was significantly attenuated when NT-proBNP was included in the model. Ky et al. implemented a jackknife approach for creation of a risk score with multiple biomarkers in a multicenter cohort of 1513 patients with chronic HF and evaluated its ability to classify risk compared to SHFS, following in principle an internal validation approach.(28) The biomarker score increased the predictive power of their model and significantly improve discrimination. However, since their biomarker model

included BNP and CRP, it is difficult to establish the impact of other biomarkers on model performance. In addition, while they internally validated their model, they performed their variable selection and model estimation on the same population, potentially arriving at overly optimistic estimates.

Many of the parameters included in current prognostic models of HF reflect the symptoms and results of disease deterioration, rather than the causes. This is the case for among other EF, NYHA-class, and to some extent may also be the case for NT-proBNP and troponins. While independence from these variables is important when considering the potential usefulness of a new clinical prognostic biomarker, this is not as evident when using biomarker studies as an approach to further understand the development of the disease. Thus, a multi-marker approach to study HF patients could be useful even if it does not improve on current prognostic models. It could still lead to new ways to categorize HF patients, and potentially aid in therapy selection. Although the present study was not designed to show this, subgroups of patients with a particular inflammatory and fibrotic phenotype could potentially benefit from a particular, targeted therapy (i.e., personalized therapy). After all, choice of therapy is not only a question of how likely a patient is to die, but also about how that patient is likely to respond to treatment. In other words, markers identifying a therapeutic target may not necessarily be markers independently predicting prognosis e.g. if a marker identified a cause of symptoms as opposed to disease progression.

Anti-inflammatory and anti-fibrotic effects are frequently referred to as some of the beneficial pleiotropic influences statins may exert on progression of cardiovascular disease including HF. While both the CORONA and GISSI-HF trials revealed a 20-30% reduction in CRP with rosuvastatin (9), we found very modest anti-inflammatory effects when evaluating a range of more specific markers of inflammation and ECM remodeling, including upstream inducers of CRP (e.g. IL-6).(5) However, many of the beneficial effects of statins on inflammatory markers in the literature are derived from populations with atherosclerotic disease and the inflammatory mechanisms that promote plaque progression and progression of myocardial failure may be somewhat different. A meta-analysis including 10 RCT's (also including CORONA and GISSI-HF) with varying etiologies support the effect of statins on more "atherogenic markers" such as CRP and VCAM-1, while no effect was found

on IL-6 and TNF.(34) Our findings suggest that the anti-inflammatory effect of statins may play a limited role in systolic ischemic HF. Furthermore, in contrast to CRP, where a beneficial statin effect was observed in patients with high levels, statin therapy improved certain outcomes in patients with low levels of several of these markers including mediators involved in fibrosis and ECM remodeling such as galectin-3 and biglycan as well as markers reflecting vascular inflammation such as OPG and PTX3. A similar treatment pattern has been observed for NT-proBNP.(35) Thus, the benefit of rosuvastatin in the lower tertiles of our models' PS may suggest that a low inflammatory burden reflects patients with lesser degree of maladaptive remodeling and fibrosis with a modifiable disease course and greater gain of statins for their underlying ischemic heart disease. Conversely, a higher score may reflect patients with irreversible tissue remodeling

There are several important limitations to our study. First, our findings may not apply to populations with different demographics, in particular HF patients with other etiologies, or HF with preserved ejection fraction. In fact, our group of patients reflects a rather homogenous and selected group of HF patients and it is possible that a multimarker approach that includes inflammatory and ECM markers could be more relevant in heterogeneous real-life HF population. Second, we have tried to avoid "over optimism" in our estimates. However, our findings are not externally validated, and investigations in similar populations may give other results In general, external validation help avoid too optimistic evaluation of models by assuring that the model is not dependent on the specific composition of the study's population to perform well.(36)This is especially the case with a rather homogenous population as in this study. However, as our main findings are negative, further decreasing the power of our models would not have changed our main conclusions. Third, we have used two approaches to model building in this paper, and both have some important drawbacks. For Model 1, all the methods applied have limitations, and the final model might not be the "perfect" model. For Model 2, variables selected are only based on the experience of the authors, and other biomarkers could have been chosen as well. We have attempted to make a model reflecting current knowledge on biomarkers in HF including what we thought was the most promising biomarkers. However, other biomarkers not measured in CORONA could increase the predictive powers of the models, as our studies have focused in inflammatory and ECM related proteins. Especially markers

such as GDF-15 and Copeptin have shown promising results, and could have improved our model further.(37,38) Fourth, not all variables used for SHFS were available in our dataset. Several of these markers (e.g., hemoglobin, lymphocyte count and uric acid) have an inflammatory component (lymphocyte counts and uric acid) or may be modified by inflammation (hemoglobin) and may partly interact with our inflammatory biomarkers (39-41) and sodium could partly be reflected by natriuretic peptides.(42) However, uric acid may also reflect mechanisms important in HF progression that are lacking in our panels such oxidative stress.(43) Still, the improvement in the model's performance when adding NT-proBNP and biomarkers to the SHFS could partially be explained by the lack of these variables and the full SHFS would probably do better compared to the CORONA score than our findings suggest.

In this study, we have investigated whether two panels of biomarkers improved prognostic abilities of a risk score built on the CORONA population, and the SHFS. We found that, while there was some improvement in discriminatory power of the models, the gains were modest and clinical relevance doubtful. Our findings do not support the notion that adding biomarkers representing different aspects of HF pathology improves the prognostic abilities of existing risk scores. We cannot, however, exclude that other panels of biomarkers or similar panels of biomarkers in other more heterogeneous HF population would give different results. We also found no correlation between changes in inflammatory and ECM-related biomarkers and treatment with rosuvastatin, suggesting that statin treatment in this population has limited anti-inflammatory effects. There was, however, a tendency for patients with lower biomarkers scores at baseline to have beneficial effects of rosuvastatin treatment.

Clinical perspectives

Previous studies have suggested that panels of multiple biomarkers may add prognostic information to established predictive metrics in CHF. However, we were not able to do this using several biomarkers previously suggested in literature, reflecting different aspects of inflammation and remodeling in HF.

Our finding suggests that beside NT-proBNP, very few biomarkers are able to add significantly to already existing risk models, even when putting them together into biomarker panels.

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Figure legends

Figure 1. Categories of biomarkers in heart failure.

Biomarkers available in the CORONA cohort sorted by categories suggested by Braunwald et. al. Since the functions of some markers are multiple, single biomarkers may appear in several categories.

Figure 2. The CORONA cohort (n=5011) is divided into a biomarker population (n=1497) and no marker population (n=3514). **2.** The biomarker population is randomly divided into a validation cohort (n=744) and a training set (n=753). **3.** From the no marker population, a CORONA predictions score (PS) is calculated from existing variables based on an established prediction model from this cohort (see methods). A Seattle Heart Failure Score PS (SHFS PS) is also calculated. **4.** In the training set 2 models are established to identify multimarker. Model 1 is selected by statistical methods in this population, while Model 2 PS is composed of variables select from the literature. **5.** A Model 1 PS and Model 2 PS is calculated based on Model 1 and Model 2 in additions to the CORONA PS. A Model 1 PS and Model 2 PS score is also calculated using the SHFS PS as a base. **6.** Model 1 PS and Model 2 PS are evaluated in the validation set by calibration and discrimination of the models.

Figure 3. Prognostic power of Model 1 or 2 with the CORONA PS or SHFS with NT-proBNP (full models) compared with only CORONA PS or SHFS with NT-proBNP respectively (limited models). Discrimination tests of difference between full and limited models, coefficients of regression model in validation sample. CV: cardiovascular, N: Number of patients, Coef: coefficient, CI: 95% confidence interval, G&B GOF test: Groennesby and Borgan goodness of fit test. K: Gönen and Heller's K, NRI: Net reclassification Index, C: Harrell's C.

Figure 4. Observed versus expected number of events by tertile of prognostic score of model 1 and 2 Number of observed events *vs.* estimated number of events by time for each tertile of the prognostic risk scores of model 1 and 2 for all-cause mortality.

Figure 5. Forest plot of treatment effect for rosuvastaint by tertile (T1-T3) of prognostic score of each model for each endpoint. Interaction p-values for Model 1 (M1) and Model 2 (M2) are given in italic on the right side of the table

Supplementary data

Supplementary methods

Patients

The design and principal findings of CORONA have been reported elsewhere in detail.(1) In brief, patients ≥60 years with chronic HF-REF of ischemic cause, in New York Heart Association (NYHA) class II–IV and with left ventricular ejection fraction (LVEF) ≤40% (≤35% if NYHA II), were eligible, provided the investigator determined that they did not need treatment with a cholesterol-lowering drug. Criteria for exclusion included recent cardiovascular (CV) events, current or planned procedures or surgery; acute or chronic liver disease or alanine aminotransferase ≥2× the upper limit of normal (ULN); serum creatinine ≥2.5 mg/dL; chronic muscle disease, contraindication to statin therapy or an unexplained creatine kinase ≥2.5× ULN; thyroid stimulating hormone ≥2× ULN; and any condition substantially reducing life expectancy.

Study outcome and definition

Definition and adjudication of all outcomes have been described in detail previously.(1) The primary outcome was a composite of CV mortality, non-fatal myocardial infarction and stroke. In addition, secondary endpoints included in the present study were all-cause mortality and CV mortality.

Biochemical sampling and analysis

All blood samples were non-fasting and all reported measurements, except for the cytokines, were made using fresh samples at a central laboratory (Medical Research Laboratories, Zaventem, Belgium). The eGFR was calculated according to the Modified Diet in Renal Disease formula.(2) Blood samples for the measurement of the cytokines were collected in pyrogen-free tubes without any additives, and serum was stored at –80ºC until analyses. All samples were thawed ≤ 3 times. NT-proBNP was analyzed using commercially available assay (Roche Diagnostics, Basel, Switzerland). An immunonephelometric method was used to measure C-reactive protein (Dade Behring, Atterbury, UK; sensitivity, 0.04 mg/L). Methods and details on the biomarkers including analytical performance are described elsewhere .(3-11) In brief, interleukin (IL)-8, IL-6, tumor necrosis factor (TNF) and

monocyte chemotractant protein (MCP1) were measured using a multiplex cytokine high-sensitivity assay (Randox Laboratories Ltd, Crumlin, Co. Antrim, UK), CCL21, YKL40, CXCL16, secreted frizzled related protein (SFRP)-3, neutrophil gelatinase-associated lipocalin (NGAL), osteoprotegerin (OPG), soluble TNF receptor type 1 (sTNFR1) and sTNFR2, and sGP130 were measured using enzyme immunoassays (EIA) from R&D systems (Wiesbaden, Germany). Biglycan, mimecan, and endostatin were measured by EIA (Roche Diagnostics, Penzberg, Germany). Galectin 3 and ST2 (Presage ST2 Assay, Critical Diagnostics, San Diego, CA, USA) were measured at BG Medicine (Waltham, MA, USA). Pentraxin 3 (PTX3) was measured by EIA (Perseus Proteomics, Tokyo, Japan). Intra- and inter-assay coefficients of variation were <10% for all assays except IL-8, TNF and MCP-1 which were < 20%.

Statistical analysis

Difference in distribution of variables between the training and validation data set were tested with Student t test for normally distributed baseline variables, Fisher exact test for categorical data, and Wilcoxon rank-sum test for non-normally distributed variables. Wilcoxon rank-sum test were also used for analysis of change in biomarkers according to treatment.

We used multiple imputation to avoid problems with missing values for several of the biomarkers, doing 20 imputations with a chained regression method for estimation of missing values. All prognostic scores were computed using the imputed dataset (See table S2). The CORONA population was divided into three sub-groups. Subgroup one had no biomarker data and was used for fitting a Cox model using routine clinical and biochemical variables as previously reported (history of diabetes, LVEF, BMI, NYHA class, ApoB/ApoA-1 ratio, history of intermittent claudication, gender, age, heart rate and eGFR, CRP and NT-proBNP).(1) The model was then used to calculate a PS by multiplication of estimated coefficients with corresponding variables for each individual subject in the biomarker population. Seattle HF score (SHFS) was calculated based on the available data by multiplication of the natural log of published hazard ratios with corresponding variables.(12) As sodium levels, lymphocyte count, as well as hemoglobin levels and uric acid were not available in the CORONA dataset, these where excluded from our SHFS.

The remaining 1497 patients were randomly divided into two subgroups; one training set and

one validation set (See table S1 for demographics and differences between these two populations). All subsequent model building and calculation of PSs based on these were done in the training data set, while testing of models' discrimination and calibration was done in the validation dataset.

To compensate for weaknesses of automatic variable selection procedures, three different approaches for were used; a stepwise selection based on minimizing Akaike information criterion (AIC), a stepwise selection procedure setting the p-value limit for inclusion to 0.2, and exclusion to 0.15, and finally using the algorithm suggested by Hosmer et al, coined purposeful variable selection (PVS).(13) For this approach p-value for inclusion was set to 0.2, for exclusion to 015, and variables were kept in the model if exclusion lead to a larger than 0.25 relative change in any of the remaining coefficients. For model selection, all biomarkers measured were separated into groups based on function as judged by authors to keep the number of events to variable ratio of the models high (Table S2). The selection algorithms where first run for each group, and finally on variables selected from each group, all models controlling for the CORONA PS. The model using selected variables were then built by examining the three models from the different variable selection algorithms, and including all common variables in the final model. In addition, all variables selected by two approaches were also added to the final model (Model 1).

The stability of the selected models was examined using a bootstrapping method randomly selecting patients from the training set and doing all three selection methods on the bootstrapped population. Frequencies of inclusion of all variables by each method were recorded and analyzed to assure no variables where frequently selected by several methods but not included in the final model. For each marker included in the model, the proportional hazards assumption was visually judged using plot of Schnell residuals, for fit using martingale residuals plotted against time, and finally checked for effect of outliers by plotting standardized difference of beta (DF-beta) values *vs.* time. In addition, one model was based on variables suggested in recent literature, consisting mainly of variables that have consistently been suggested to give prognostic information for patients with chronic HF. (7,14-19) A PS was finally calculated by fitting these variables using a Cox proportional hazard model in the training set, and predicting the PS for patients in the validation dataset controlling for the CORONA PS.

To evaluate the different models, the discrimination of the PS from each model was compared to PSs from only the CORONA risk model, or SHFS with and without NT-proBNP using Harrell's C-statistics, Gönen and Heller's K statistics. Calibration was tested by examining the coefficient of the PS in a regression model in the validation population, which should be close to one if the risk slope is approximately equal as in the training population. Formal tests of calibration as a Groennesby and Borgan test of calibration and visual inspection of Arjas like plots of observed versus predicted risk in tertiles of PSs were also done.

To test interaction between models and effect of statin treatment, a PS of only biomarkers included in model 1 and 2 was calculated, and a formal interaction test with rosuvastatin treatment was conducted adjusting for the CORONA PS. The p-value of the interaction is reported. In addition, the effect of treatment in each tertile of this PS was evaluated by testing treatment in each tertile of model 1 and 2 PS, adjusted for the full model 1 and 2, including the CORONA PS. All calculations were done using STATA 14.0 (StataCorp LP, College Station, TX, USA). P-values <0.05 were considered significant, except for interaction tests where <0.1 was considered significant.

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Table S1. Patient characteristics of participants in the CORONA sub-study

	Non-biomarker population (n=3514)	Biomarker population (n=1497)	Training population (n=753)	Verification population (n=744)	P-value Training vs verification	P-value biomarkers vs non-biomarkers
Age, y	73±7	72±7	72±7	72±7	0.625	<0.001
Female sex, n (%)	832 (23)	348 (23)	180 (24)	168 (23)	0582	0.771
NYHA class, n II/III/IV	1379/2079/56	478/1002/17	245/501/7	233/501/10	0.670	<0.001
Ejection fraction	0.31±0.06	0.32±0.07	0.32±0.07	0.32±0.07	0.893	<0.001
BMI kg/m^2	27.18±4.5	27.3±4.6	27.1±4.5	27.4±4.7	0.323	0.630
Systolic BP, mm Hg	129±16	130±16	130±16	129±16	0.084	0.383
Diastolic BP, mm Hg	76±9	77±9	77±9	77±9	0.109	<0.001
Hear rate, beats/min	72± 11	71±11	71±11	71±11	0.608	0.002
Current smoker, n (%)	348 (10)	180 (12)	87 (12)	93 (13)	0.579	0.027
Medical history, n (%)						
Myocardial infarction	2061 (59)	945 (63)	476 (63)	469 (63)	0.957	0.003
CABG or PCI	912 (26)	317 (21)	157 (21)	160 (22)	0.800	<0.001
Hypertension	2140 (61)	1035 (69)	521 (69)	514 (69)	1.000	<0.001
Diabetes mellitus	1089 (31)	388 (26)	194 (26)	194 (26)	0.906	<0.001
Atrial fibrillation	865 (24)	329 (22)	172 (23)	157 (21)	0.418	0.046
Stroke	443 (13)	181 (12)	91 (12.	90 (12)	1.000	0.640
Intermittent claudication	475 (14)	162 (11)	82 (11)	80 (11)	0.934	0.009
COPD	80 (3)	29 (2)	15 (2)	14 (2)	1.000	0.526
Laboratory measurements						
Total cholesterol, m*M*	5.15±1.07	5.23±1.09	5.22±1.12	5.25±1.06	0.637	0.010
LDL_ cholesterol, m*M*	3.51±0.92	3.65±0.98)	3.65±1.06	3.65±0.90	0.949	<0.001
HDL_ cholesterol, m*M*	1.23±0.35	1.23±0.34	1.23±0.35	1.24±0.34	0.663	0.935

Triglycerides, m*M*	2.00±1.23	2.01±1.39	2.03±1.53	1.99±1.22	0.660	0.780
ApoA-1/ApoB ratio	0.87±0.25	0.89±0.25	0.89±0.26	0.89±0.24	0.864	0.007
eGFR, (mL/min/1.73^2 BSA)	56±14	57±14	58±14	57±14	0.556	0.293
NT-proBNP, p*M*	177 (79-377)	160 (60-343)	177 (73-345)	143 (53-343)	0.066	0.003
CRP, mg/L	3.4 (1.5-7.3)	3.7 (1.6-7.8)	3.7 (1.6-8.0)	3.7 (1.6-7.6)	0.966	0.044
Current medication, n (%)						
Loop or tiazide	2844 (81)	1133 (76)	560 (74)	573 (77)	0.456	<0.001
Aldosterone antagonist	1364 (39)	542 (36)	271 (36)	271 (36)	0.872	0.086
ACE inhibitor	2773 (79)	1208 (81)	607 (81)	601 (81)	0.948	0.158
ARB	486 (14)	151 (10)	79 (11)	72 (10)	0.608	<0.001
β-blocker	2586 (74)	1136 (76)	586 (78)	550 (74)	0.080	0.090
Digitalis glycoside	1187 (34)	431 (29)	226 (30)	205 (28)	0.304	0.001
Number of deaths	1050	437	205	232	0.062	0.636

Categorical data are reported as n (percentages) and continuous data as mean±SD or for CRP and NT-proBNP as median and 25th and 75th percentile. Conversion factor for NT-proBNP: 1 pmol/L=8.457 pg/mL. ACE indicates angiotensin-converting enzyme; ApoA-I, apolipoprotein I; ApoB, apolipoprotein B; ARB, angiotensin receptor blocker; BMI, body mass index; CABG, coronary artery bypass grafting; ; CRP, C-reactive protein; eGFR, estimated glomerular filtration rate; HDL, high-density lipoproteinLDL, low-density lipoprotein; NT-proBNP, amino-terminal pro-brain natriuretic peptide; NYHA, New York Heart Association; PCI, percutaneous coronary intervention.

Table S2 Groups of biomarkers measured with missing observations.

ECM/Fibrosis	Chemokines	Cardiac stress	Vascular inflammation	Cytokines
Biglycan (96)	IL 8 (17)	ST2 (48)	OPG (36)	sTNFR1 (73)
Mimecan (204)	MCP1(17)	TnT (252)	NGAL (82)	sTNFR2 (94)
Endostatin (106)	CxCL16 (32)	SFRP3 (53)	PTX3 (40)	IL 6 (17)
YKL40 (158)	CCL21 (41)			sGP130 (45)
Galectin 3 (35)				TNF (17)

Number of missing observation in parenthesis. ECM: Extra-cellular matrix, IL: Interleukin, MCP1: Monocyte chemotactic protein 1, CxCL16: Chemokine ligand 16, CCL21: Chemokine ligand 21, sST2: Soluble ST2, TnT: Troponin T, SFRP3: secreted frizzled-related protein 3, OPG: Osteoprtegerin, NGAL: Neutrophil gelatinase-associated lipocalin, PTX 3: Pentraxin 3, sTNFR: soluble tumor necrosis factor receptor, sGP130: soluble glycoprotein 130

Table S3. Model coefficients

	Model 1			Model 2		
Variables	Coef (95% CI)	*p-value*	t	Coef (95% CI)	*p-value*	t
IL 8 (log)	0.22 (0.06–0.37)	*0.01*	2.73			
Galectin 3 (log)	-0.05 (-0.49–0.40)	*0.83*	-0.21	0.08 (-0.36–0.53)	*0.72*	0.36
TnT (log)	0.22 (0.00–0.44)	*0.05*	1.98	0.25 (0.03–0.46)	*0.02*	2.36
sST2 (log)	0.27 (-0.02–0.55)	*0.06*	1.85	0.22 (-0.06–0.51)	*0.12*	1.5
CCL21 (log)	0.20 (-0.04–0.44)	*0.10*	1.65			
Endostatin (log)	0.20 (-0.21–0.61)	*0.33*	0.97			
PTX3 (log)				-0.09 (-0.33–0.16)	*0.49*	-0.68
sTNFR2 (log)				0.05 (-0.19,0.29)	*0.68*	0.42
CORONA PS*	0.80 (0.55–1.04)	*<0.001*	6.47	0.86 (0.61-1.10)	*<0.001*	6.95
N	753			753		
Events	205			205		

IL: Interleukin, CCL21: Chemokine ligand 21, sST2: Soluble ST2, TnT: Troponin T, PTX3: Pentraxin 3, sTNFR2: soluble tumor necrosis factor receptor 2, log: Natural log transformed.

Table S4. Prognostic power of model 1 and 2 *vs.* the CORONA PS

	N/Events	Coef (CI) t	G&B GOF test:	K limited/full	Delta K (CI)	NRI(NRIe/NRIne)	C limited/full	Delta C (CI)
All-cause mortality								
Model 1	744/232	**1.13** (0.97 - 1.29) 13.94		0.725/0.731	0.006 (-0.027–0.038)	**0.23 (0.01/0.22)**	0.747/0.754	0.007 (-0.007-0.021)
p-value		*<0.001*	*0.842*		*0.842*	*0.014*		*0.303*
Model 2	744/232	**1.22** (1.05–1.387) 14.02		0.725/0.733	0.008 (-0.025–0.040)	**0.27 (0.08/0.19)**	0.747/0.755	0.008 (-0.003-0.019)
p-value		*<0.001*	*0.753*		*0.642*	*0.004*		*0.163*
CV mortality								
Model 1	744/196	**1.16** (0.99–1.34) 12.87		0.730/0.735	0.005 (-0.029–0.039)	0.16 (-0.02/0.18)	0.756/0.761	0.005 (-0.010-0.020)
p-value		*<0.001*	*0.324*		*0.781*	*0.159*		*0.513*
Model 2	744/196	**1.245** (1.06–1.43) 13.20		0.730/0.736	0.006 (-0.028–0.040)	**0.26 (0.09/0.17)**	0.756/0.760	0.005 (-0.007-0.017)
p-value		*<0.001*	*0.301*		*0.731*	*0.012*		*0.437*
Primary Endpoint								
Model 1	744/233	**1.04** (0.89–1.20) 13.01		0.713 /0.719	0.006 (-0.029–0.040)	**0.23 (0.03/0.20)**	0.728/0.736	0.008 (-0.007-0.022)
p-value		*<0.001*	*0.736*		*0.740*	*0.023*		*0.303*
Model 2	744/233	**1.14** (0.97–1.31) 13.18		0.713 /0.723	0.010 (-0.024–0.045)	**0.34 (0.12/0.23)**	0.728/0.739	0.010 (-0.001-0.022)
p-value		*<0.001*	*0.778*		*0.557*	*<0.001*		*0.073*

Prognostic power of Model 1 or 2 with the CORONA PS compared with only CORONA PS. N: Number of patients, Coef: Coefficients, CI: 95% confidence interval, G&B GOF test: Groennesby and Borgan goodness of fit test, lim. Only CORONA PS, full: CORONA PS and model 1 or model 2. K: Gönen and Heller's K, NRI: Net reclassification Index, NRIe: Component NRI for events, NRIne: Component NRI for non-events, C: Harrell's C. Statistically significant results in bold.

Table S5. Prognostic power of Model 1 and 2 *vs.* the SHFS and NT-proBNP

		Coef (CI) t	G&B GOF test:	K limited/full	Delta K (CI)	NRI (NRIe/NRIne)	C limited/full	Delta C
All-cause mortality								
Model 1	744/232	**1.11** (0.95 - 1.26) 13.80		0.713/0.722	0.009 (-0.025–0.042)	**0.35 (0.10/0.25)**	0.726/0.744	0.018 (-0.003-0.037)
p-value		*<0.001*	*0.440*		*0.602*	*<0.001*		*0.066*
Model 2	744/232	**1.19** (1.02–1.354) 13.09		0.713/0.723	0.010 (-0.024–0.043)	**0.34 (0.12/0.22)**	0.726/0.742	0.015 (-0.002-0.033)
p-value		*<0.001*	*0.276*		*0.564*	*<0.001*		*0.066*
CV mortality								
Model 1	744/196	**1.15** (0.98–1.32) 13.14		0.721/0.727	0.006 (-0.029–0.041)	**0.30 (0.08/0.22)**	0.738/0.752	0.013 (-0.009-0.034)
p-value		*<0.001*	*0.689*		*0.733*	*0.006*		*0.217*
Model 2	744/196	**1.22** (1.04–1.40) 13.20		0.721/0.727	0.007 (-0.028–0.041)	**0.32 (0.12/0.20)**	0.738/0.749	0.010 (-0.008-0.029
p-value		*<0.001*	*0.687*		*0.715*	*0.001*		*0.256*
Primary Endpoint								
Model 1	744/233	**1.01** (0.85–1.16) 12.63		0.694 /0.707	0.013 (-0.023–0.049)	**0.34 (0.10/0.24)**	0.701/0.723	**0.022 (0.002-0.043)**
p-value		*<0.001*	*0.287*		*0.472*	*0.001*		*0.031*
Model 2	744/233	**1.09** (0.93–1.26) 12.82		0.694 /0.710	0.016 (-0.020–0.052)	**0.42 (0.17/0.25)**	0.701/0.724	**0.023 (0.007-0.043)**
p-value		*<0.001*	*0.356*		*0.373*	*<0.001*		*0.009*

Prognostic power of Model 1 or 2 with SHFS and NT-proBNP compared with only SHFS and NT-proBNP. SHFS: Seattle heart failure score, NT-proBNP: N-terminal pro brain natriuretic peptide. N: Number of patients, Coef: Coefficient, CI: 95% confidence interval, G&B GOF test: Groennesby and Borgan goodness of fit test, K: Gönen and Heller's K, NRI: Net reclassification Index, NRIe: Component NRI for events, NRIne: Component NRI for non-events, C: Harrell's C. Statistically significant results in bold.

Table S6. Model 1 and 2 vs. the SHFS risk score excluding NT-proBNP

	N/Events	Coef (CI) t	G&B GOF test:	K limited/full	Delta K (CI)	NRI (NRIe/NRIne)	C limited/full	Delta C
All-cause mortality								
Model 1	726/228	**1.04** (0.87 - 1.22) 11.85		0.570/0.692	**0.122** (0.079–0.165)	**0.69 (0.27/0.42)**	0.578/0.714	**0.137 (0.080-0.164)**
p-value		*<0.001*	*0.190*		*<0.001*	*<0.001*		*<0.001*
Model 2	726/228	**1.20** (1.00–1.40) 11.80		0.570/0.693	**0.123** (0.080–0.166)	**0.66 (0.28/0.38)**	0.578/0.709	**0.131 (0.091-0.175)**
p-value		*<0.001*	*0.878*		*<0.001*	*<0.001*		*<0.001*
CV mortality								
Model 1	726/192	**1.06** (0.87–1.25) 11.07		0.571/0.694	**0.124** (0.077–0.170)	**0.69 (0.27/0.42)**	0.576/0.718	**0.142 (0.091-0.181)**
p-value		*<0.001*	*0.245*		*<0.001*	*<0.001*		*<0.001*
Model 2	726/192	**1.22** (1.01–1.44) 11.07		0.571/0.696	**0.125** (0.079–0.172)	**0.68 (0.32/0.35)**	0.576/0.713	**0.137 (0.072-0.162**
p-value		*<0.001*	*0.729*		*<0.001*	*<0.001*		*<0.001*
Primary Endpoint								
Model 1	726/228	**0.98** (0.81–1.15) 11.16		0.549 /0.683	**0.134** (0.090–0.179)	**0.65 (0.27/0.38)**	0.554/0.700	**0.146 (0.104-0-188)**
p-value		*<0.001*	***0.033***		*<0.001*	*<0.001*		*<0.001*
Model 2	726/228	**1.18** (0.97–1.37) 13.18		0.549 /0.690	**0.141** (0.097–0.186)	**0.65 (0.29/0.36)**	0.554/0.700	**0.146 (0.105-0.189)**
p-value		*<0.001*	*0.850*		*<0.001*	*<0.001*		*<0.001*

SHFS: Seattle heart failure score. Limited: Only SHFS, full: SHFS and model 1 or 2. N: Number of patients, Coef: Coefficients, CI: 95% confidence interval, G&B GOF test: Groennesby and Borgan goodness of fit test, K: Gönen and Heller's K, NRI: Net reclassification Index, NRIe: Component NRI for events, NRIne: Component NRI for non-events, C: Harrell's C. Statistically significant results in bold.

Table S7. Change in biomarkers after 3 months of rosuvastatin or placebo treatment

	Baseline		3 Months		Relative Change		
Variable	Ros.	Placebo	Ros.	Placebo	Ros.	Placebo	*p-value*
Biglycan, ng/mL	**18**	**17**	**19**	**18**	**0.528**	**0.429**	***0.046***
Mimecan, ng/mL	62	63	64	64	0.521	0.065	*0.648*
Endostatin, ng/mL	169	171	170	172	0.039	0.028	*0.801*
YKL40, ng/mL	**216**	**214**	**208**	**222**	**0.041**	**0.147**	***0.001***
Galectin 3, ng/mL	21	20	20	20	0.029	0.013	*0.591*
IL-8, pg/mL	7.8	12.1	8.0	12.3	0.628	0.648	*0.902*
MCP1, pg/mL	209	208	208	208	0.066	0.057	*0.811*
CXCL16, pg/mL	**1110**	**1100**	**1059**	**1090**	**-0.012**	**0.027**	***0.002***
CCL21, pg/mL	692	699	731	675	0.096	0.178	*0.657*
sST2, ng/mL	21.1	20.7	22.2	21.9	0.093	0.112	*0.802*
TnT µg/mL	18.9	19.2	19.8	19.0	0.196	0.117	*0.400*
SFRP3, ng/mL	1.67	1.69	1.67	1.62	0.147	0.096	*0.413*
OPG, ng/mL	4.98	4.97	5.00	5.00	0.030	0.025	*0.999*
NGAL, ng/mL	345	348	347	352	0.087	0.096	*0.345*
PTX3, ng/mL	**7.04**	**6.71**	**7.58**	**6.96**	**0.162**	**0.121**	***0.000***
sTNFR1, ng/mL	2.25	2.27	2.30	2.34	0.108	0.172	*0.230*
sTNFR2, ng/mL	0.26	0.26	0.26	0.27	0.200	0.133	*0.109*
IL-6, pg/mL	6.06	5.63	5.97	7.55	0.358	0.966	*0.887*
sGP130, ng/mL	362	362	358	362	0.004	0.018	*0.296*

P-value for difference in change between placebo and rosuvastatin treatment. IL: Interleukin, MCP1: Monocyte chemotactic protein 1, CXCL16: Chemokine ligand 16, CCL21: Chemokine ligand 21, sST2: Soluble ST2, TnT: Troponin T, SFRP3: secreted frizzled-related protein 3, OPG: Osteoprtegerin, NGAL: Neutrophil gelatinase-associated lipocalin, PTX 3: Pentraxin 3, Ros.: Rosuvastatin; sTNFR: soluble tumor necrosis factor receptor, sGP130: soluble glycoprotein 130

Table S8. Change in markers according to 3 month statin treatment in the top 2 tertiles.

	Baseline		3 Months		Relative Change		
Variable	Ros.	Placebo	Ros.	Placebo	Ros.	Placebo	*p-value*
Biglycan	24.8	23.5	25.0	23.6	0.083	0.048	0.135
Mimecan	77	79	77	78	0.010	0.000	0.810
Endostatin, ng/mL	200	199	195	196	-0.007	-0.006	0.935
YKL40, ng/mL	**274**	**286**	**256**	**282**	**-0.032**	**0.038**	**0.007**
Galectin 3, ng/mL	24.0	23.3	23.2	22.8	-0.021	-0.010	0.621
IL 8, pg/mL	10.8	17.3	9.8	16.3	0.395	0.451	0.887
MCP1, pg/mL	250	252	236	242	-0.034	-0.006	0.506
CXCL16, pg/mL	**1283**	**1283**	**1174**	**1210**	**-0.074**	**-0.044**	**0.038**
CCL21, pg/mL	895	894	925	818	0.015	-0.015	0.941
ST2, ng/mL	26.5	25.8	27.0	27.0	0.021	0.078	0.686
TnT	26.4	26.7	26.5	25.9	0.048	0.051	0.913
SFRP3, ng/mL	2.15	2.16	2.07	2.01	0.001	-0.050	0.503
OPG, ng/mL	5.92	5.93	5.79	5.84	-0.015	-0.011	0.880
NGAL, ng/mL	422	443	404	424	-0.006	-0.022	0.188
PTX3, ng/mL	**8.90**	**8.23**	**9.12**	**8.26**	**0.071**	**0.053**	**0.001**
sTNFR1, ng/mL	2.77	2.87	2.69	2.64	0.019	-0.014	0.797
sTNFR2, ng/mL	0.33	0.34	0.32	0.33	-0.018	-0.002	0.781
IL 6, pg/mL	8.69	7.95	8.00	10.42	0.202	1.134	0.969
sGP130, ng/mL	416	413	400	403	-0.034	-0.018	0.120

Ros: Rosuvastatin, IL: Interleukin, MCP1: Monocyte chemotactic protein 1, CXCL16: Chemokine ligand 16, CCL21: Chemokine ligand 21, sST2: Soluble ST2, TnT: Troponin T, SFRP3: secreted frizzled-related protein 3, OPG: Osteoprtegerin, NGAL: Neutrophil gelatinase-associated lipocalin, PTX 3: Pentraxin 3, sTNFR: soluble tumor necrosis factor receptor, sGP130: soluble glycoprotein 130.