



Limited Added Value of Circulating Inflammatory Biomarkers in Chronic Heart Failure

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ABSTRACT

OBJECTIVES This study sought to evaluate whether a panel of biomarkers improved prognostication in patients with heart failure (HF) and reduced ejection fraction of ischemic origin using a systematized approach according to suggested requirements for validation of new biomarkers.

BACKGROUND Modeling combinations of multiple circulating markers could potentially identify patients with HF at particularly high risk and aid in the selection of individualized therapy.

METHODS From a panel of 20 inflammatory and extracellular matrix biomarkers, 2 different biomarker panels were created and added to the Seattle HF score and the prognostic model from the CORONA (Controlled Rosuvastatin Multinational Trial in Heart Failure) study (n = 1,497), 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—model 1 (endostatin, interleukin 8, soluble ST2, troponin T, galectin 3, and chemokine [C-C motif] ligand 21) and model 2 (troponin T, soluble ST2, galectin 3, pentraxin 3, and soluble tumor necrosis factor receptor 2)—significantly improved the CORONA and Seattle HF models but added only modestly to their Harrell's C statistic and net reclassification index. In addition, rosuvastatin had no effect on the levels of a wide range of inflammatory and extracellular matrix markers, but there was a tendency for patients with a lower level of biomarkers in the 2 panels to have a positive effect from statin treatment.

CONCLUSIONS In the specific HF patient population studied, a multimarker approach using the particular panel of biomarkers measured was of limited clinical value for identifying future risk of adverse outcomes. (J Am Coll Cardiol HF 2017;5:256-64) © 2017 by the American College of Cardiology Foundation.

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Manuscript received September 1, 2016; revised manuscript received January 9, 2017, accepted January 21, 2017.

The prognosis in patient with 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 previously evaluated a range of biomarkers in CORONA (Controlled Rosuvastatin Multinational Trial in Heart Failure) study, comprising elderly patients with moderate-to-severe ischemic HF (3-16). Classifying these markers according to categories proposed by Braunwald (2) revealed good coverage of the different pathological pathways activated in HF (Figure 1), with a focus on inflammation and matrix remodeling. 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 statistic (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.

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Although measurements of individual markers of inflammation and the EMC so far have not improved 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 a full description of the methods, see the Online Appendix. A flow chart of the statistical approach is shown in Figure 2. Briefly, the CORONA population was divided into 3 subgroups. Subgroup 1 had no

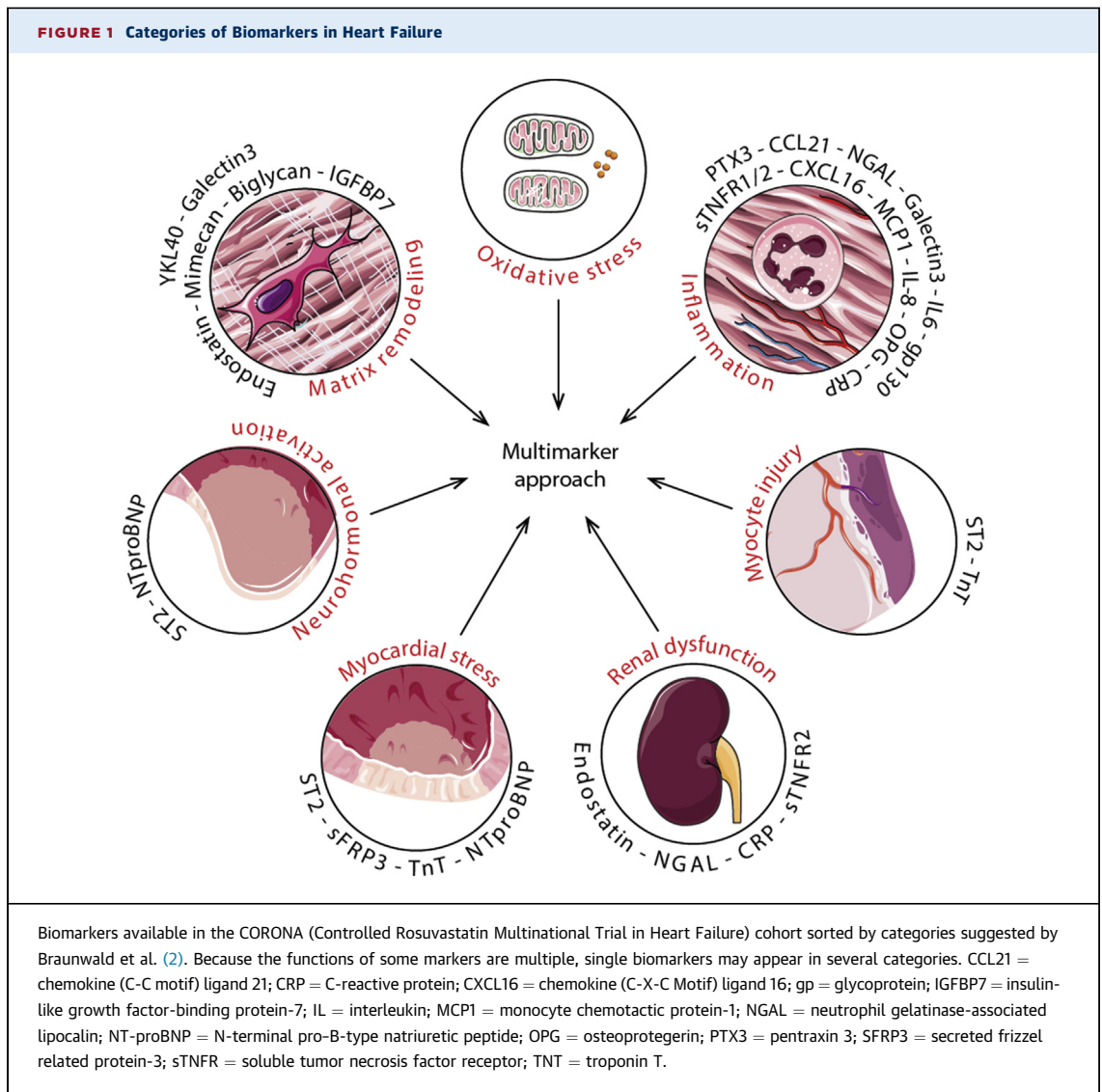
biomarker data and was used for fitting a Cox model including routine clinical and biochemical variables as previously reported (history of diabetes, left ventricular ejection fraction, body mass index, New York Heart Association functional class, apolipoprotein B/apolipoprotein A-1 ratio, history of intermittent claudication, gender, age, heart rate, estimated glomerular filtration rate, C-reactive protein (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. The Seattle heart failure score (SHFS) was calculated based on the available data (22). Because sodium levels, lymphocyte count, and hemoglobin and uric acid acids were not available in the CORONA dataset, they were excluded from our SHFS.

RESULTS

MODEL BUILDING. Demographics of the CORONA inflammatory substudy and the training and validation set are given in Online Table 1. 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, that is, biglycan, mimecan, endostatin, YKL40, galectin-3, interleukin (IL)-8, monocyte chemotactic protein (MCP)-1, Chemokine (C-X-C motif) ligand 16 (CXCL16), chemokine (C-C motif) ligand-21, soluble ST2 (sST2), troponin T (TnT), secreted frizzled-related protein-3 (SFRP3), osteoprotegerin (OPG), neutrophil gelatinase-associated lipocalin (NGAL), pentraxin-3 (PTX3), soluble tumor necrosis factor receptor (sTNFR)-1, sTNFR2, IL-6, soluble glycoprotein-130, and tumor necrosis factor. The 3 different approaches to building a model from available biomarkers yielded 3 slightly different results. By keeping all variables as proposed by at least 2 methods, 6 variables remained in model 1: endostatin, IL-8, sST2, TnT, galectin-3, and chemokine (C-C motif) ligand 21 (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 (see Online Table 2). For model 2, we included more established HF risk markers from the literature: TnT, sST2, galectin-3, PTX3, and sTNFR2 (Table 1) (7,23-27).

ABBREVIATIONS AND ACRONYMS

CRP	= C-reactive protein
CV	= cardiovascular
ECM	= extracellular matrix
HF	= heart failure
IL	= interleukin
NRI	= net reclassification index
NT-proBNP	= N-terminal pro-B-type natriuretic peptide
PS	= prognostic score
PTX3	= pentraxin 3
SHFS	= Seattle heart failure score
sTNFR	= soluble tumor necrosis factor receptor
sST2	= soluble ST2
TNT	= troponin T



PERFORMANCE OF THE MULTIMARKER MODELS. The PS values 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 2 models were added to the CORONA PS, the models showed reasonable calibration by a Groennesby and Borgan test score (Figure 3, Online Table 3), 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 Online Table 4.

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, Online Table 3). However, the addition of each biomarker model led 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 an event getting a lower risk score (Online Table 3).

COMPARISON WITH SHFS. 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 (Online Table 5). 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, Online Table 6).

EFFECT OF STATIN TREATMENT ON MARKERS OF INFLAMMATION.

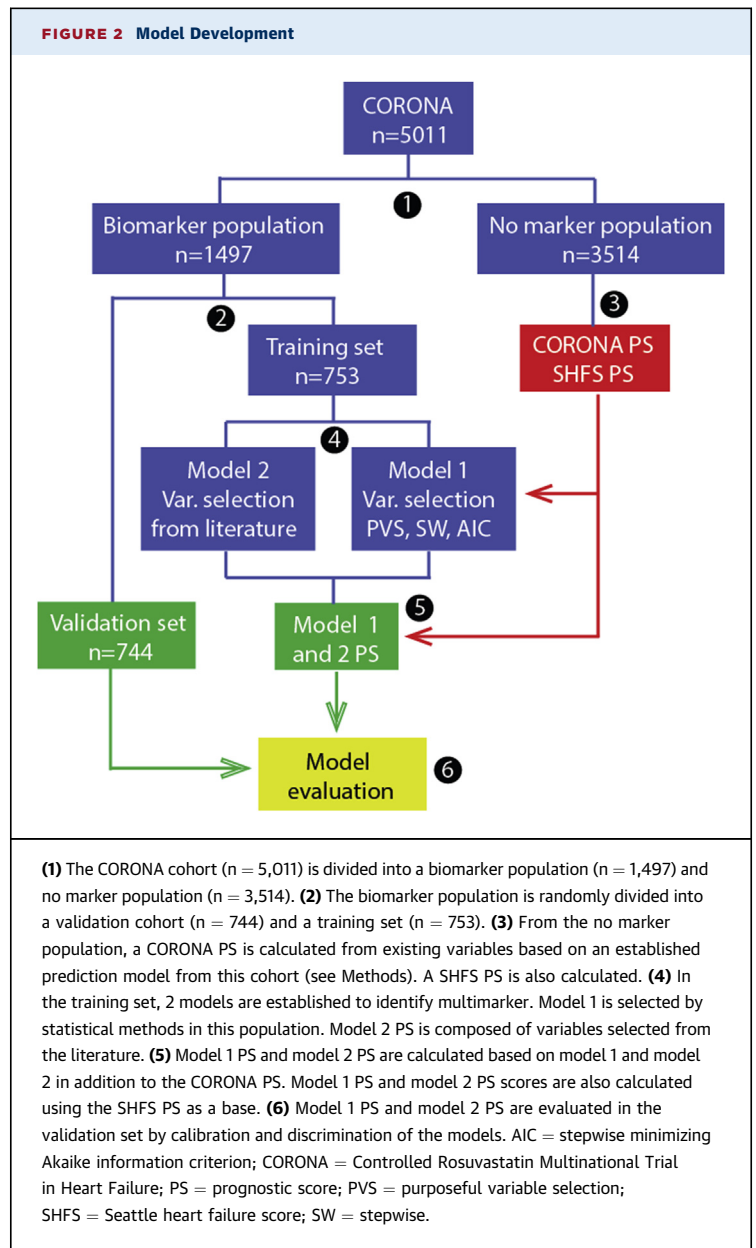
In the CORONA trial, patients were randomly assigned to treatment with rosuvastatin or placebo. Therefore, we were able to investigate whether 3 months of statin treatment influenced biomarker levels in patients with HF. As shown in Online Table 7, the relative change in biomarker levels differed between the treatment arms only for biglycan, YKL40, CXCL16, and PTX3. Biglycan and PTX3 increased more in the rosuvastatin group, whereas CXCL16 and YKL40 increased more in the placebo group. Because patients with low levels of biomarkers may have a limited potential for benefiting from the anti-inflammatory effect of statins, we also assessed treatment effects in the top 2 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 observed (Online Table 8).

EFFECT OF STATIN TREATMENT IN DIFFERENT RISK GROUPS.

Finally, we evaluated the interaction between the PS of models 1 and 2, and the 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, which was not the case for any of the other patients. We obtained similar results when testing interaction for model 2 PS, suggesting that patients with little inflammatory activity at the baseline of study had some effect from 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. Although 2 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 is uncertain. Furthermore, when comparing the 2 models to the previously published CORONA model, there was only a small but significant NRI. Thus, although 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 it does suggest that patients with a lower inflammatory burden may benefit from statin therapy.

We used 2 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' judgment of biomarkers that

TABLE 1 Variable Selection and Model Building

Variable	SW	PVS	SW AIC	CORONA	Model 1	Model 2
Endostatin	X	X	X		X	
IL-8	X	X			X	
sST2	X	X			X	X
TnT	X	X	X		X	X
Galectin-3	X	X	X		X	X
CCL21	X		X		X	
sGP130	X					
sTNFR2						X
PTX3						X
Wald score				183	136	132
LR				189	138	134
Harrell's C				0.747	0.714	0.707
K				0.725	0.695	0.695
p Difference					0.087	0.092

Variables selected by each automatic bivariable selection approach, as well as variables finally included in models 1 and 2. Performance of each model alone compared with CORONA prognostic score.

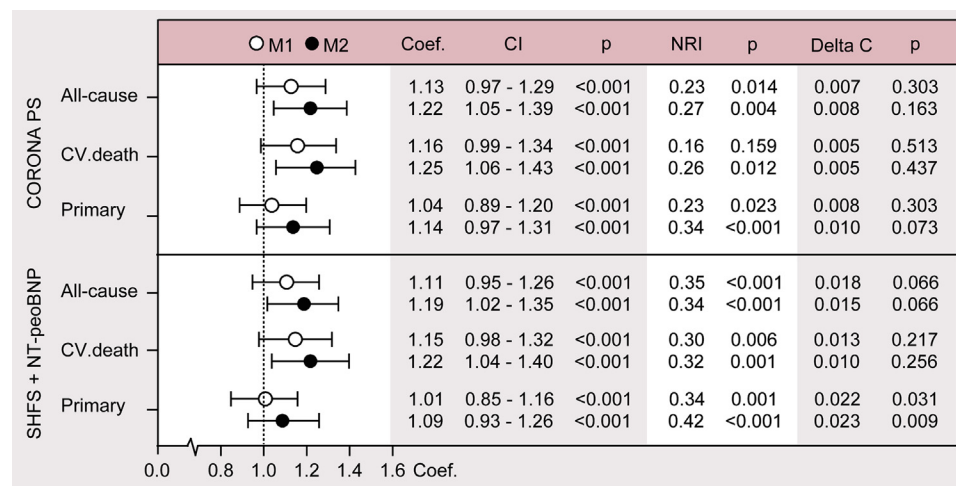
CCL21 = chemokine (C-C motif) ligand 21; CORONA = Controlled Rosuvastatin Multinational Trial in Heart Failure; IL = interleukin; K = Gönen and Heller's K; LR = likelihood ratio; PTX3 = pentraxin 3; PVS = purposeful variable selection; sGP130 = soluble glycoprotein 130; sST2 = soluble ST2; sTNFR2 = soluble tumor necrosis factor receptor 2; SW = stepwise; SW AIC = stepwise minimizing Akaike information criterion; TnT = troponin T.

have repeatedly been suggested or have been shown to be associated with outcome in several previous studies and were 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). Although 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.

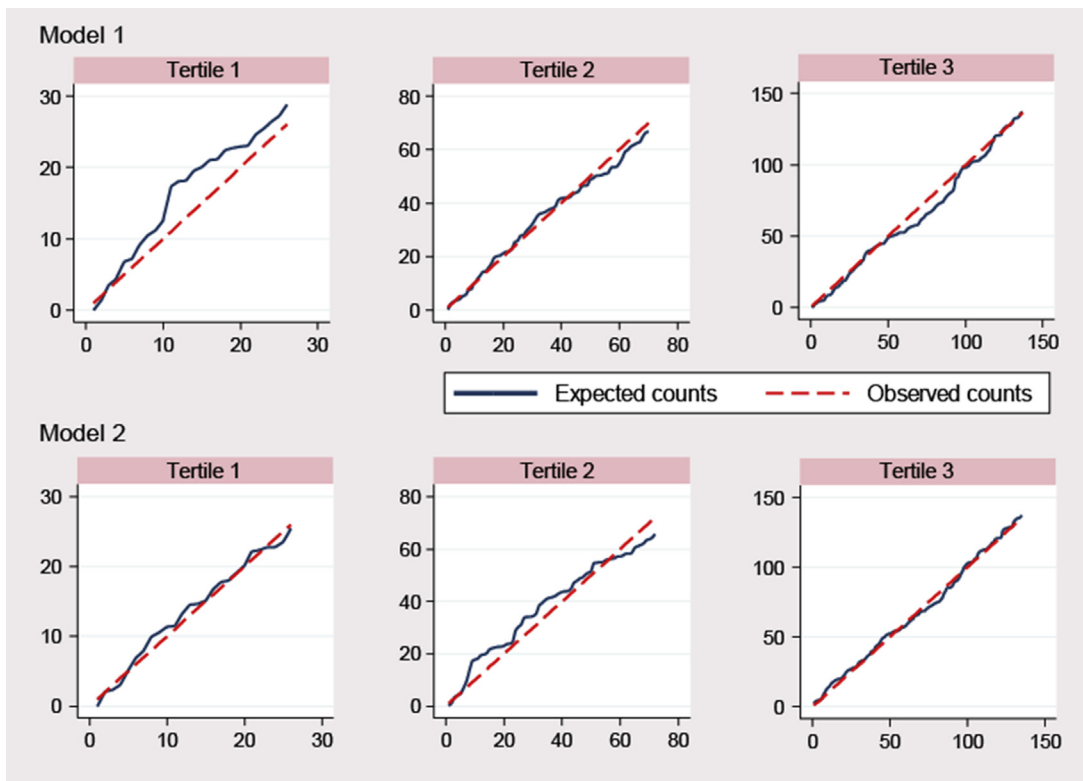
Measures such as NRI and C statistics may be used for quantification of the usefulness of a new biomarker, but what may be considered clinically significant changes of these measurements is still an open question (32). Furthermore, the lack of statistical significance of these measures, and in particular 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 on the order of 0.02, and we believe that smaller changes would give little clinical meaning. In addition, NRI and C statistics are overly optimistic when applied to

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. C = Harrell's C statistic; CI = 95% confidence interval; Coef = coefficient; CV = cardiovascular; M1 = model 1; M2 = model 2; NRI = net reclassification index; other abbreviations as in Figures 1 and 2.

FIGURE 4 Observed Versus Expected Number of Events by Tertile of Prognostic Score of Models 1 and 2



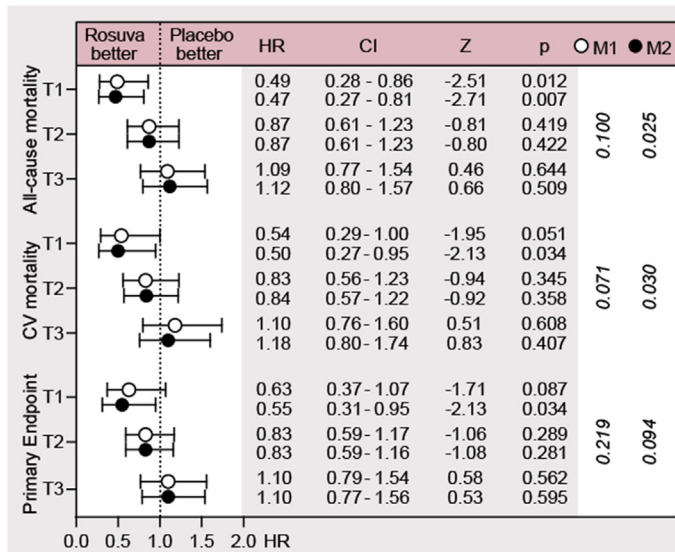
Number of observed events versus estimated number of events by time for each tertile of the prognostic risk scores of models 1 and 2 for all-cause mortality.

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, although 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. (28) implemented a jackknife approach for creation of a risk score with multiple biomarkers in a multicenter cohort of 1,513 patients with chronic HF and evaluated its ability to classify

risk compared to SHFS, following in principle an internal validation approach. The biomarker score increased the predictive power of their model and significantly improve discrimination. However, because their biomarker model included BNP and CRP, it is difficult to establish the impact of other biomarkers on model performance. In addition, although 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 EF and New York Heart Association functional class, and, to some extent, may also be the case for NT-proBNP and troponins. Although 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

FIGURE 5 Forest Plot of Treatment Effect for Rosuvastatin by Tertile (T1-T3) of Prognostic Score of Each Model for Each Endpoint

Interaction p values for model 1 (M1) and model 2 (M2) given in **italics on the right side of the table**. HR = hazard ratio; other abbreviations as in [Figure 3](#).

multimarker approach to study patients with HF could be useful even if it does not improve on current prognostic models. It could still lead to new ways to categorize patients with HF 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 antifibrotic effects are frequently referred to as some of the beneficial pleiotropic influences statins may exert on progression of CV disease, including HF. Although both the CORONA and GISSI-HF (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico-Heart Failure) trials revealed a 20% to 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 reported 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 of 10 randomized controlled trials (including CORONA and GISSI-HF) with varying etiologies support the effect of statins on more “atherogenic markers” such as CRP and vascular cell adhesion molecule 1, whereas no effect was found on IL-6 and tumor necrosis factor (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, for which 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 degrees 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.

STUDY LIMITATIONS. First, our findings may not apply to populations with different demographics, in particular patients with HF of other etiologies or HF with preserved ejection fraction. In fact, our group of patients reflects a rather homogeneous and selected group of patients with HF, and it is possible that a multimarker approach that includes inflammatory and ECM markers could be more relevant in a heterogeneous real-life HF population. Second, we attempted to avoid “overoptimism” 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 in order to perform well (36). This is especially the case with a rather homogeneous population as in this study. However, because our main findings are negative, further decreasing the power of our models would not have changed our main conclusions. Third, we used 2 approaches to model building in this study, 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 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 on inflammatory and ECM-related proteins. In particular, markers such as Growth Differentiation Factor 15 (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 they may be modified by inflammation (hemoglobin) and may partly interact with our inflammatory biomarkers (39-41). 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 as oxidative stress (43). Still, the improvement in the model’s performance when NT-proBNP and biomarkers are added to the SHFS could partially be explained by the lack of these variables, and the full SHFS probably would do better compared to the CORONA score than suggested by our findings.

CONCLUSIONS

In this study, we investigated whether 2 panels of biomarkers improved the prognostic abilities of a risk score built on the CORONA population and the SHFS. We found that although 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. However, we cannot exclude that other panels of biomarkers or similar panels of biomarkers in other more heterogeneous HF populations 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 biomarker scores at baseline to have beneficial effects of rosuvastatin treatment.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: 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 accomplish this using several biomarkers previously suggested in literature, reflecting different aspects of inflammation and remodeling in HF.

TRANSLATIONAL OUTLOOK: Our finding suggests that other than NT-proBNP, few biomarkers are able to add significantly to already existing risk models, even when they are put together into biomarker panels.

REFERENCES

1. Mann DL. Innate immunity and the failing heart: the cytokine hypothesis revisited. *Circ Res* 2015; 116:1254-68.
2. Braunwald E. Heart failure. *J Am Coll Cardiol* 2013; 61:1-20.
3. Askevold ET, Nymo S, Ueland T, et al. Soluble glycoprotein 130 predicts fatal outcomes in chronic heart failure: analysis from the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA). *Circ Heart Fail* 2013; 6:91-8.
4. Ueland T, Nymo SH, Latini R, et al. CCL21 is associated with fatal outcomes in chronic heart failure: data from CORONA and GISSI-HF trials. *Eur J Heart Fail* 2013; 15:747-55.
5. Nymo SH, Hulthe J, Ueland T, et al. Inflammatory cytokines in chronic heart failure: interleukin-8 is associated with adverse outcome. Results from CORONA. *Eur J Heart Fail* 2014; 16:68-75.
6. Gullestad L, Ueland T, Kjekshus J, et al. The predictive value of galectin-3 for mortality and cardiovascular events in the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA). *Am Heart J* 2012; 164:878-83.
7. Broch K, Ueland T, Nymo SH, et al. Soluble ST2 is associated with adverse outcome in patients with heart failure of ischaemic aetiology. *Eur J Heart Fail* 2012; 14:268-77.
8. Nymo SH, Ueland T, Askevold ET, et al. The association between neutrophil gelatinase-associated lipocalin and clinical outcome in chronic heart failure: results from CORONA. *J Intern Med* 2012; 271:436-43.
9. Latini R, Gullestad L, Masson S, et al. Pentraxin-3 in chronic heart failure: the CORONA and GISSI-HF trials. *Eur J Heart Fail* 2012; 14: 992-9.
10. Ueland T, Dahl CP, Kjekshus J, et al. Osteoprotegerin predicts progression of chronic heart failure: results from CORONA. *Circ Heart Fail* 2011; 4:145-52.
11. Ueland T, Aukrust P, Nymo SH, et al. Novel extracellular matrix biomarkers as predictors of adverse outcome in chronic heart failure: association between biglycan and response to statin therapy in the CORONA trial. *J Card Fail* 2015; 21: 153-9.
12. Askevold ET, Gullestad L, Nymo S, et al. Secreted frizzled related protein 3 in chronic heart

- failure: analysis from the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA). *PLoS One* 2015;10:e0133970.
13. Gravning J, Askevold ET, Nymo SH, et al. Prognostic effect of high-sensitive troponin T assessment in elderly patients with chronic heart failure: results from the CORONA trial. *Circ Heart Fail* 2014;7:96-103.
 14. Dahl CP, Aukrust P, Nymo SH, et al. Prognostic value of CXCL16 in patients with left ventricular systolic dysfunction and heart failure. *Int J Cardiol* 2013;168:4427-9.
 15. Gullestad L, Ueland T, Kjekshus J, et al. Galectin-3 predicts response to statin therapy in the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA). *Eur Heart J* 2012;33:2290-6.
 16. Ueland T, Aukrust P, Nymo SH, et al. Predictive value of endostatin in chronic heart failure patients with poor kidney function. *Cardiology* 2015;130:17-22.
 17. Ahmad T, Fiuzat M, Pencina MJ, et al. Charting a roadmap for heart failure biomarker studies. *J Am Coll Cardiol HF* 2014;2:477-88.
 18. Richter B, Koller L, Hohensinner PJ, et al. A multi-biomarker risk score improves prediction of long-term mortality in patients with advanced heart failure. *Int J Cardiol* 2013;168:1251-7.
 19. Jungbauer CG, Riedlinger J, Block D, et al. Panel of emerging cardiac biomarkers contributes for prognosis rather than diagnosis in chronic heart failure. *Biomark Med* 2014;8:777-89.
 20. Collins GS, Reitsma JB, Altman DG, Moons KG. Transparent reporting of a multivariable prediction model for individual prognosis or diagnosis (TRIPOD): the TRIPOD statement. *BMJ* 2015;350:g7594.
 21. Wedel H, McMurray JJV, Lindberg M, et al. Predictors of fatal and non-fatal outcomes in the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA): incremental value of apolipoprotein A-1, high-sensitivity C-reactive peptide and N-terminal pro B-type natriuretic peptide. *Eur J Heart Fail* 2009;11:281-91.
 22. Levy WC, Mozaffarian D, Linker DT, et al. The Seattle heart failure model: prediction of survival in heart failure. *Circulation* 2006;113:1424-33.
 23. Bayes-Genis A, Richards AM, Maisel AS, Mueller C, Ky B. Multimarker testing with ST2 in chronic heart failure. *Am J Cardiol* 2015;115:76B-80B.
 24. Gaggin HK, Szymonifka J, Bhardwaj A, et al. Head-to-head comparison of serial soluble ST2, growth differentiation factor-15, and highly-sensitive troponin T measurements in patients with chronic heart failure. *J Am Coll Cardiol HF* 2014;2:65-72.
 25. Braunwald E. Biomarkers in heart failure. *N Engl J Med* 2008;358:2148-59.
 26. Hartupee J, Mann DL. Positioning of inflammatory biomarkers in the heart failure landscape. *J Cardiovasc Transl Res* 2013;6:485-92.
 27. Bozkurt B, Mann D, Deswal A. Biomarkers of inflammation in heart failure. *Heart Fail Rev* 2010;15:331-41.
 28. Ky B, French B, Levy WC, et al. Multiple biomarkers for risk prediction in chronic heart failure. *Circ Heart Fail* 2012;5:183-90.
 29. Ketchum ES, Levy WC. Establishing prognosis in heart failure: a multimarker approach. *Prog Cardiovasc Dis* 2011;54:86-96.
 30. Vistnes M, Christensen G, Omland T. Multiple cytokine biomarkers in heart failure. *Exp Rev Mol Diagn* 2010;10:147-57.
 31. Altman DG, Royston P. What do we mean by validating a prognostic model? *Stat Med* 2000;19:453-73.
 32. Leening MJ, Vedder MM, Wittteman JC, Pencina MJ, Steyerberg EW. Net reclassification improvement: computation, interpretation, and controversies: a literature review and clinician's guide. *Ann Intern Med* 2014;160:122-31.
 33. Allen LA, Felker GM. Multi-marker strategies in heart failure: clinical and statistical approaches. *Heart Fail Rev* 2010;15:343-9.
 34. Zhang L, Zhang S, Jiang H, et al. Effects of statin therapy on inflammatory markers in chronic heart failure: a meta-analysis of randomized controlled trials. *Arch Med Res* 2010;41:464-71.
 35. Cleland JG, McMurray JJ, Kjekshus J, et al. Plasma concentration of amino-terminal pro-brain natriuretic peptide in chronic heart failure: prediction of cardiovascular events and interaction with the effects of rosuvastatin: a report from CORONA (Controlled Rosuvastatin Multinational Trial in Heart Failure). *J Am Coll Cardiol* 2009;54:1850-9.
 36. Bleeker SE, Moll HA, Steyerberg EW, et al. External validation is necessary in prediction research: a clinical example. *J Clin Epidemiol* 2003;56:826-32.
 37. Balling L, Gustafsson F. Copeptin in heart failure. *Adv Clin Chem* 2016;73:29-64.
 38. Wollert KC, Kempf T. Growth differentiation factor 15 in heart failure: an update. *Curr Heart Fail Rep* 2012;9:337-45.
 39. Leyva F, Anker SD, Godsil IF, et al. Uric acid in chronic heart failure: a marker of chronic inflammation. *Eur Heart J* 1998;19:1814-22.
 40. Okonko DO, Mandal AK, Missouri CG, Poole-Wilson PA. Disordered iron homeostasis in chronic heart failure: prevalence, predictors, and relation to anemia, exercise capacity, and survival. *J Am Coll Cardiol* 2011;58:1241-51.
 41. Vaduganathan M, Greene SJ, Butler J, et al. The immunological axis in heart failure: importance of the leukocyte differential. *Heart Fail Rev* 2013;18:835-45.
 42. Kalra PR, Anker SD, Coats AJ. Water and sodium regulation in chronic heart failure: the role of natriuretic peptides and vasopressin. *Cardiovasc Res* 2001;51:495-509.
 43. Alcaïno H, Greig D, Chiong M, et al. Serum uric acid correlates with extracellular superoxide dismutase activity in patients with chronic heart failure. *Eur J Heart Fail* 2008;10:646-51.

KEY WORDS biomarkers, chronic ischemic heart disease, heart failure, inflammation, mortality, survival

APPENDIX For an expanded Methods section and supplemental tables, please see the online version of this paper.