Low YKL-40 in Chronic Heart Failure may predict beneficial effects of statins: Analysis from the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA)

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Abstract

Context and objective: To evaluate if YKL-40 can provide prognostic information in patients with ischemic heart failure (HF) and identify patients who may benefit from statin therapy. **Materials and Methods:** The association between serum YKL-40 and predefined outcome was evaluated in 1344 HF patients assigned to rosuvastatin or placebo. **Results:** YKL-40 was not associated with outcome in adjusted analysis. In YKL-40 tertile 1, an effect on the primary outcome (HR 0.50, p=0.006) and CV death (HR 0.54 p=0.040) was seen by rosuvastatin in adjusted analysis. **Conclusions:** A beneficial modification of outcome was observed with statin therapy in patients with low YKL-40 levels.

Clinical Trial Registration Information: URL: http://www.clinicaltrials.gov. Unique identifier: NCT00206310.

Introduction

Despite major therapeutic advances over the past three decades, the prognosis for patients with heart failure (HF) remains poor and the need for new treatments remain apparent (Braunwald 2013;Mosterd et al. 2007). Biomarkers may help in developing new treatments either by targeting therapies to patients at highest risk or by identifying specific pathophysiological pathways responsible for disease progression (Bartunek 2009;Dalzell et al. 2014). YKL-40 is a plasma glycoprotein primarily secreted by activated macrophages, but may also be produced by neutrophils, vascular smooth muscle cells and chondrocytes in the presence of inflammation (Johansen 2006;Kastrup 2012). Although the precise functions of YKL-40 are not identified, it is suggested to play a role in inflammation, fibrosis and extracellular matrix (ECM) remodeling (Johansen 2006;Volck et al. 1998). Several studies have demonstrated increased systemic levels of YKL-40 in patients with ischemic heart disease (Harutyunyan et al. 2013;Kastrup et al. 2009;Kucur et al. 2007;Michelsen et al. 2010;Wang et al. 2008), with particularly high levels following acute myocardial infarction (MI), and these have been correlated with disease progression and severity (Hedegaard et al. 2010;Kucur et al. 2007).

In a cross sectional study Mygind et al. demonstrated lower serum levels of YKL-40 in statin treated, compared with non-statin treated, coronary artery disease patients (Mygind et al. 2011), although the effect of a statin on YKL-40 levels has not been examined in a placebo-controlled randomized controlled trial. Recently, in a large cohort (n=717) of HF patients with mixed etiology, Harutyunyan et al. reported that elevated levels of serum YKL-40 were associated with all-cause mortality (Harutyunyan et al. 2012).

Based on the emerging importance of YKL-40 in the progression of ischemic heart disease,

including its role in inflammation, fibrosis and ECM remodeling, we hypothesized that YKL-40 could predict adverse outcomes in patients with ischemic HF. This was assessed in the Controlled Rosuvastatin Multinational Trial in Heart Failure (CORONA) population, a contemporary cohort of older patients with chronic systolic ischemic HF randomly assigned to statin therapy (rosuvastatin) or placebo in a double-blind fashion (Kjekshus et al. 2007). Our goals were to determine whether: i) YKL-40 provides independent prognostic information in this population ii) statins regulate YKL-40 levels and iii) YKL-40 levels could be used to identify a subgroup of patients who may benefit from statin therapy

Methods

Patients and Study Procedures

The design and principal findings of CORONA have been reported in detail (Kjekshus et al. 2007). Clinical Trial Registration Information: URL: http://www.clinicaltrials.gov. Unique identifier: NCT00206310. Briefly, patients ≥60 years of age with chronic HF attributed to ischemic heart disease, defined as (i) medical history or ECG signs of MI or (ii) other data suggesting an ischemic etiology (e.g. wall motion disturbances on echocardiography or history of other occlusive atherosclerotic disease [i.e. earlier stroke, intermittent claudication, percutaneous coronary intervention (PCI)]), who were in New York Heart Association (NYHA) class II-IV, with a LV ejection fraction (LVEF) ≤40% (≤35% if NYHA II), were eligible for inclusion. Patients were randomly assigned to rosuvastatin 10 mg/day or matching placebo, once-daily. The trial complied with the Declaration of Helsinki and was approved by the Ethics Committees of the participating hospitals. All patients provided written informed consent. Ethics committee/institutional review board: Regional Etiksprövningskommitten I Göteborg, Sahlgrenska Akademin, Mediniargatan 3, Plan 5. Diary number: Ö284-03. The name of the ethics committees from any of the participating

hospitals can be provided on request. Name of study locations can be found in (Askevold et al. 2015). The present study was an optional, predefined sub-study of the main CORONA trial conducted at 378 participating hospitals, focusing on inflammatory biomarkers, which included patients from centers capable of collecting the necessary blood samples. Compliance, side-effects and dropouts in the CORONA trial have been reported previously (Kjekshus et al. 2007).

Study outcomes and definitions

The primary predefined outcome was the composite of death from cardiovascular (CV) causes, non-fatal MI, and non-fatal stroke, analyzed as time to the first event. The secondary predefined outcomes were (analyzed as time to first event) a) all-cause mortality, b) CV mortality (including cause-specific CV death), c) coronary endpoint (defined as sudden death, fatal or non-fatal MI, performance of PCI or coronary artery bypass graft surgery [CABG], ventricular defibrillation by an implantable cardioverter-defibrillator [ICD], resuscitation from cardiac arrest, or hospitalization for unstable angina pectoris), d) the number of hospitalizations for CV causes, and e) hospitalization for worsening HF (WHF). The definition and adjudication of all outcomes have been described in detail previously, as have data on C-reactive protein (CRP) and N-terminal pro-B-type natriuretic peptide (NT-proBNP) (Cleland et al. 2009;Kjekshus et al. 2007;McMurray et al. 2009;Wedel et al. 2009).

Blood sampling and biochemical analyses

YKL-40 was measured from blood samples taken after an overnight fast. All other blood samples were non-fasting and analyzed on fresh samples at a central laboratory (Medical Research Laboratories, Zaventem, Belgium). NT-proBNP was analyzed using commercially available assay (Roche Diagnostics, Basel, Switzerland). An immunonephelometric high-sensitivity method was used to measure CRP (Dade Behring, Atterbury, UK; sensitivity 0.04

mg/L). Serum YKL-40 was measured by enzyme immunoassay (R&D Systems, Minneapolis, MN) with intra- and inter- assay coefficients of variation <10%. To validate this assay, another commercial assay from Quidel (Quidel, San Clara, CA) was used.

Statistical analysis

For comparing two groups, the Mann-Whitney U test was used. Kaplan-Meier curves were constructed to visualize and evaluate (log rank test) differences in survival. Trends across tertiles of YKL-40 were tested using the Cuzick extension of the Wilcoxon rank-sum test. A restricted cubic spline (RCS) analysis with three knots was undertaken on the outcome all-cause mortality to assess linearity of risk. Survival analyses were performed using the Cox proportional hazard regression models to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) for YKL-40 included as log-transformed standardized (per standard deviation) continuous variables at baseline or as nominal changes in a version of the model developed previously for the full CORONA population (Wedel et al. 2009), which included mainly clinical variables at step one (LVEF, NYHA class, age, body mass index [BMI], diabetes mellitus [DM], sex, intermittent claudication, and heart rate [HR]). At step two, estimated glomerular filtration rate (eGFR) and apolipoprotein (Apo) B/ApoA-1 ratio were included in the model, and finally, at stage 3, the log-transformed serum concentrations of NT-proBNP and CRP were included. Harrel's C-statistic was calculated for all endpoints using the full model with and without YKL-40, and the difference between the C-statistics was estimated. We implemented a jack-knife cross-validation approach to correct for over-optimism associated with validating a model in the same material from which it is developed. In this approach predictions for each observation were obtained from models developed on the remaining observations. These cross-validated probabilities were used to calculate jack-knife C-statistics. Calculation of the net reclassification improvement (NRI) is increasingly being

used to evaluate the prognostic usefulness of a biomarkers (Pencina et al. 2010). When no established risk categories exist, the use of a category-free NRI has been advocated (Pencina et al. 2011). We therefore calculated the category-free NRI after adding YKL-40 to the full model. Confidence intervals and p-values for NRI were determined by boot-strapping with 2000 repetitions. A two-sided p-value <0.05 was considered to be significant, except for interaction terms, for which p-values <0.10 were accepted (Zelen 1971). All statistical analyses were performed using STATA version 11 for Windows (StataCorp, College Station, TX).

Results

Of the 5,011 patients enrolled in the CORONA study, a measurement of YKL-40 levels was available in 27% (n=1344). Compared with the entire CORONA population, the patients in the present study were slightly younger, were more often in NYHA class III, more frequently had a history of myocardial infarction and hypertension and had a higher mean LVEF, diastolic blood pressure, and cholesterol level. Conversely, the proportion with diabetes mellitus and a pacemaker was smaller (Table 1). Patient characteristics according to tertile values of YKL-40 are shown in Table 1. Patients with higher serum levels of YKL-40 were more likely to be older and have a lower BMI, diastolic blood pressure and total- and LDL cholesterol. They were also more likely to have atrial fibrillation and worse kidney function. NT-proBNP and CRP concentrations were significantly higher in patients with higher YKL-40 levels. Stepwise linear regression identified higher age (per decade β±SE 28.5±6.1, p<0.001), NT pro-BNP (log 19.6±3.3, p<0.001) and CRP levels (log 26.3±3.4, p<0.001), and lower total cholesterol concentration (-8.4±3.9, p=0.029), as the strongest predictors of YKL-40 levels.

Association between serum YKL-40 levels and clinical outcomes

During a median follow-up of 955 (817, 1103) days, 396 patients died. Kaplan-Meier plots for the primary end point, all-cause- and CV mortality and the coronary endpoint revealed a poorer outcome for patients in the two top tertiles of YKL-40 compared to the bottom tertile (Figure 1). Unadjusted Cox proportional hazard regression models displayed significant associations between log-transformed standardized baseline YKL-40 (log/SD) levels and all the endpoints (Table 2). These associations were moderately attenuated but significant for all outcome after adjustment for demographics and baseline characteristics (Step 1, e.g. LVEF, NYHA, age, BMI, diabetes) and traditional risk markers (Step 2: ApoB/ApoA-1 ratio and eGFR) except sudden death. However, after adjusting for NT-proBNP and CRP (Step 3), the association between YKL-40 and all outcome variables was markedly attenuated and not significant. The primary endpoint in adjusted analysis without NT-proBNP and CRP but with YKL40 had a c-statistic of 0.68, while with NT-proBNP and CRP but without YKL40, the C-statistic is 0.72 ($p<0.001$). Use of diuretics ($p=0.66$) or digitalis ($p=0.20$) had no impact on the primary endpoint.

Change in levels of YKL-40 levels during rosuvastatin treatment

A small increase in YKL-40 levels was observed in the placebo group (median [25^{th}, 75^{th} percentile]: baseline 169 [110,288] and 3 months 183 [113,279] $p=0.017$), while a minor decrease was found during rosuvastatin treatment (baseline 180 [117,291] and 3 months 175 [111,274] $p=0.053$) resulting in a modest but significant difference in change from baseline between the treatment arms (placebo: +4 [-28,46] vs. rosuvastatin: -3 [-47,34] $p=0.002$). However, the change in YKL-40 was not related to outcome (data not shown). Statins decreased LDL levels as described previously (Kjekshus et al. 2007). However, there was no difference in the decrease in LDL-cholesterol across YKL-40 tertiles in either treatment arm

(p=0.84 comparing the change across tertiles).

Interactions between treatment and serum levels of YKL-40 and outcome

Interactions between treatment and serum levels of YKL-40 and outcome are depicted in Figure 2 which shows Cox adjusted placebo/rosuvastatin HRs (full adjustment including hsCRP and NT-proBNP) for each tertile of YKL-40. The interaction by treatment p-values for YKL-40 was significant for the primary endpoint, CV mortality, mortality from WHF and total mortality (with $p<0.1$ considered significant for an interaction). Further analysis revealed that while use of rosuvastatin was not associated with the primary endpoint or CV mortality in those with intermediate of high YKL-40 levels, the incidence of these endpoints was significantly reduced by rosuvastatin in tertile 1 (Figure 2). Thus, the treatment benefit for the primary endpoint in T1 of BNP (Wald 7.1, HR 0.45 (0.25-0.81) p=0.008) and T1 of YKL-40 (Wald 7.2, HR 0.50 (0.30-0.83) p=0.006) are comparable.

When including initial cholesterol levels in the fully adjusted analysis, the effect of treatment on the primary outcome (p=0.025); treatment effect in T1: HR 0.49 (0.29-0.85) p=0.011, and CV mortality (p=0.055); treatment effect in T1: HR 0.53 (0.29-0.98) p=0.044, was only marginally attenuated. As shown in Figure 3, the beneficial effect of rosuvastatin in YKL-40 tertile 1 was accompanied by a significantly larger decrease in total cholesterol, compared to tertile 3.

Comparison of the YKL-40 assay

A restricted cubic spline analysis of baseline YKL-40 concentration versus all-cause mortality confirmed the non-linearity of this relationship, with a linear increase in the first tertile, followed by flattening of the curve for tertiles 2 and 3 (Figure 4A). A previous study in patients with chronic HF of mixed etiology (n=717) demonstrated a linear increase in all-

cause mortality across quartiles of YKL-40 using a different commercial EIA (Harutyunyan et al. 2012). To evaluate potential methodological differences between the two assays we analyzed 40 random serum samples with both assays. As shown in Figure 4B, there was a strong correlation between both assays ($r=0.92$, $p<0.001$). Our EIA gave values that were 12% higher than the Quidel assay but after normalizing for this difference, a quartile analysis showed good correspondence between the two assays (Figure 4C). In an additional analysis of the relationship between YKL-40 quartile and outcomes in CORONA (Figure 4D), there was a similar pattern to that seen with tertiles, with leveling of the risk from quartile 2 upwards and no gain in predictive power with increasing quartiles. Thus, the different association with outcome between the two studies is likely not due to methodological differences.

Discussion

In this retrospective sub-study of CORONA, we found that baseline levels of YKL-40 were not associated with clinical outcomes in patients with advanced, ischemic, systolic HF in adjusted analyses. However, rosuvastatin did seem to have beneficial effects in patients with low circulating levels of YKL-40 i.e. in the lowest tertile. Thus, while baseline levels of YKL-40 may have limited use as a prognosticator in patients with HF, YKL-40 may be useful in identifying a subset of patients who may benefit from statin therapy, although this finding needs to be confirmed in a prospective controlled trial.

YKL-40 seems to be a universal marker for non-specific disease as it is increased in multiple diseases with an element of inflammation and tissue remodeling (Johansen 2006;Kastrup 2012). In a large study of the general population (n=8899), Johansen et al. demonstrated a strong association between increased circulating YKL-40 and risk of early death from CV diseases, cancer and other chronic inflammatory diseases (Johansen et al.

2010). Three previous studies have evaluated the association between YKL-40 levels and outcome in HF. In two smaller studies, Rathcke et al. (n=194) found no association with overall mortality or incident CV disease (Rathcke et al. 2010), while Bilim et al. (n=121) found that YKL-40 was an independent predictor of cardiac events (Bilim et al. 2010). However, by far the largest of these (n=717) demonstrated that YKL-40 was associated with all-cause mortality in HF patients, also after multivariable adjustment, including NT-proBNP and CRP (Harutyunyan et al. 2012). In our study YKL-40 was associated with multiple outcomes after adjustment for acknowledged clinical and some biochemical predictors. However, the associations were markedly attenuated and no longer significant following adjustment for CRP and NT-proBNP. Several recent studies demonstrate associations between circulating YKL-40 and the presence and extent of CAD (Harutyunyan et al. 2013;Kastrup et al. 2009;Kucur et al. 2007;Wang et al. 2008), and one could anticipate that YKL-40 would be a particularly strong biomarker in ischemic HF. However, the mechanisms that promote plaque progression and progression of myocardial failure may be somewhat different. Moreover, the present study population was elderly patients with severe HF, representing a rather homogenous population narrow range of several demographics including age and kidney function and exclusive ischemic etiology, potentially contributing to differences between these studies. Also, in the CORONA population, NT-proBNP has proved to be a particularly strong prognosticator and is by far the strongest predictor of outcomes in this population (Wedel et al. 2009). Thus, YKL-40 may reflect a more general age-related disease progression independent of etiology.

While rosuvastatin did not reduce the primary outcome in CORONA (Kjekshus et al. 2007) and the role of statin therapy on HF is unclear (Gastelurrutia et al. 2013;von 2009), some biomarkers in this cohort have demonstrated an interaction with statin therapy and identified subgroups with beneficial treatment effects (Cleland et al. 2009;Gullestad et al.

2012;McMurray et al. 2009;Ueland et al. 2015). Although only a minor treatment effect of statins on serum YKL-40 was observed, our study suggests that targeting HF patients with low levels of YKL-40 for statin treatment may improve certain outcomes. This is comparable to our findings for galectin-3 and the ECM proteoglycan biglycan, mediators involved in fibrosis and ECM remodeling (Gullestad et al. 2012;Ueland et al. 2015). We and others have previously shown that YKL-40 may reflect macrophage activation, tissue remodeling and fibrosis (Johansen 2006;Michelsen et al. 2010;Volck et al. 1998) which all characterize the myocardium in ischemic cardiomyopathy and are associated with elevated levels of ECM markers (Dalzell et al. 2014;Deardorff et al. 2009). Although YKL-40 is expressed in multiple tissues, it is tempting to hypothesize that low YKL-40 levels may identify patients with a modifiable disease course while patients with higher circulating levels might have such increased expression within the myocardium that they have irreversible tissue remodeling. However, as this is a clinical study and associations do not imply a causal relationship, there could be a random element in our findings. Furthermore, as NT-proBNP is a widely available standardized analysis, the clinical usefulness of these findings are at present unknown.

Certain strengths of this study include a large sample size and a large number of end points. However, for some subgroup analyses, fewer end points might explain the lack of significance, and these data should be interpreted cautiously. Moreover, the study was performed in trial patients over 60 years who have less comorbidity. Thus, the results cannot necessarily be applied to the general HF population. Also, the patients included had systolic HF and our findings might not apply to patients with preserved LVEF.

Conclusion

In conclusion, circulating levels of YKL-40 had limited predictive value in patients with chronic HF of ischemic cause. While there was only a minor effect of rosuvastatin on serum YKL-40 concentration, our results suggest that statin therapy might improve clinical outcomes in HF patients with low levels of YKL-40.

Declarations of interest

JJVM, LG, and JK were on the CORONA steering committee and have received lecture fees from AstraZeneca. JW was earlier also adviser on cardiovascular research at AstraZeneca Research Laboratories, Mölndal, Sweden. The other authors report no conflicts.

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Table 1. clinical and biochemical baseline characteristics stratified by tertile values of YKL-40

	Total Corona n=5011	Substudy n=1344	Tertile 1 (<133)	Tertile 2 (133-243)	Tertile 3 (>243)	P-trend
Age, y	72.7±7.1	71.8 ±6.9	70.0±6.5	72.5± 6.7	73.3±7.0	<0.000
Female Sex, n (%)	1180 (24)	335 (22.6)	115 (25.7)	98 (21.9)	93 (20.8)	0.080
NYHA class, n						0.068
II	1857	471 (31.8)	160 (35.7)	128 (28.6)	134 (29.9)	
III	3081	994 (67.1)	284 (63.4)	312 (69.6)	310 (69.2)	
IV	73	17 (1.1)	4 (0.9)	8 (1.8)	4 (0.9)	
Ejection Fraction	0.31±0.06	0.32±0.07	0.32±0.07	0.32±0.06	0.31±0.07	0.059
Body mass index	27.2±4.5	27.2±4.6	27.6±4.6	27.2±4.7	26.9±4.5	0.035
Systolic BP, mm Hg	129.3±4.5	130 ± 16.1	129 ±14.5	130 ±17.0	129 ±16.5	0.590
Diastolic BP, mmHg	76.2±8.9	77 ±8.9	78±8.9	77±8.8	76±9.0	0.010
Heart rate, beats per/min	71±11.2	71±10.7	71±11.2	71±11.0	71±10.1	0.217
Current smoker	528(10.5)	165 (11.1)	49 (10.9)	55 (12.3)	50 (11.2)	0.916
Medical history						
Myocardial infarction	3006 (60.0)	939 (63.4)	296 (66.1)	275 (61.4)	278 (62.1)	0.213
PCI, PTCA or CABG	1229 (24.5)	321 (21.7)	85 (19.0)	102 (22.8)	104 (23.2)	
Hypertension	3175 (63.4)	1031 (69.6)	319 (71.2)	310 (69.2)	308 (68.8)	0.424
Diabetes Mellitus	1477 (29.5)	392 (26.5)	108 (24.1)	130 (29.0)	120 (26.8)	0.365
Atrial fibrillation	1194 (23.8)	325 (21.9)	77 (17.2)	96 (21.4)	119 (26.6)	0.001
Stroke	624 (12.5)	179 (12.1)	53 (11.8)	65 (14.5)	46 (10.3)	0.475
Laboratory measurements						
Cholesterol,Mmol/L						
Total	5.2±1.1	5.2±1.09	5.3±1.07	5.2±1.07	5.1±1.08	0.000
LDL	3.6±0.9	3.7±0.98	3.8±1.01	3.6±0.93	3.5±0.92	0.000
HDL	1.2±0.35	1.2±0.35	1.2±0.33	1.2±0.34	1.2±0.37	0.834
Triglycerides, Mmol/L	2.00±1,28	2.0±1.37	1.9±1.13	2.0±1.40	2.1±1.38	0.057
ApoB/ApoA-1 ratio	0.87±0.25	0.88±0.25	0.91±0.25	0.86±0.24	0.87±0.26	0.061
eGFRMDRD	57±14.4	58±14.4	61±13.4	57± 13.1	55±15.7	0.000
NT-proBNP, pM	173(73,368)	158(61,342)	100(40,234)	185 (80,338)	206 (91,472)	0.000

CRP, mg/L	3.5 (1.6,7.4)	3.6(1.6,7.6)	2.8 (1.2,5.3)	3.8 (1.8,7.7)	4.8 (2.1,11.4)	0.000
Current medication						
Diuretic						0.010
Thiazide or loop	3977 (79.4)	1113 (75.1)	343 (76.6)	335 (74.8)	341(76.1)	
Both	357 (7,1)	169 (11.4)	40 (8.9)	47 (10.5)	60 (13.4)	
Aldosteron antagonist	1906 (38.0)	544 (36.7|)	177 (39.5)	161 (35.9)	155 (34.6)	0.127
ACE inhibitor	3981 (79.4)	1195 (80.6)	366 (81.7)	356 (79.5)	360 (80.4)	0.613
ARB	637 (12.7)	150 (10.1)	46 (10.3)	39 (8.7)	50 (11.2)	0.127
β-blocker	3722 (74.3)	1132 (76.4)	350 (78.1)	348 (77.7)	329 (73.4)	0.099
Digitalis glycoside	1618 (32.3)	433 (29.2)	112 (25.0)	126 (28.1)	146 (32.6)	0.012

NT-proBNP and CRP are displayed as median and 25^{th} and 75^{th} percentile. Other variables are shown as number (percentage of total) or as mean±SD where appropriate. P-Trend, p-value for trend across all tertiles; NYHA, New York Heart Association; BMI, body mass index; PCI, percutaneous coronary intervention; PTCA, percutaneous transluminal coronary angioplasty; CABG, coronary artery bypass grafting; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ApoB, apolipoprotein B; ApoA-1, apolipoprotein A-1; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; NT-proBNP, amino-terminal pro-brain natriuretic peptide; ACE, angiotensin converting enzyme; ARB, angiotensin II receptor blocker.

Table 2. Multivariable analysis of levels of baseline log-transformed standardized YKL-40 as a predictor of outcome in CORONA.

YKL-40	Events	HR (95% CI)	*p*-value	Wald	C index, Δ	NRI
Primary end point					0.0011 (0.045)	0.07 (0.39)
Unadjusted	383	1.30 (1.17-1.44)	0.000	24.7		
Step 1	383	1.21 (1.09-1.35)	0.000	13.1		
Step 2	382	1.18 (1.06-1.31)	0.003	9.08		
Step 3	295	1.03 (0.91-1.17)	0.613	0.26		
All-cause mortality					0.0001 (0.93)	0.09 (0.26)
Unadjusted	396	137 (1.24-1.52)	0.000	37.2		
Step 1	396	1.27 (1.14-1.41)	0.000	20.3		
Step 2	395	1.23 (1.11-1.37)	0.000	15.3		
Step 3	306	1.07 (0.95-1.21)	0.263	1.25		
CV mortality					0.0002 (0.88)	0.08 (0.34)
Unadjusted	319	1.39 (1.24-1.55)	0.000	31.8		
Step 1	319	1.28 (1.14-1.44)	0.000	17.9		
Step 2	318	1.24 (1.11-1.40)	0.000	13.3		
Step 3	245	1.10 (0.96-1.26)	0.19	1.74		
Death from WHF					0.0037 (0.29)	0.17 (0.61)
Unadjusted	98	1.61 (1.30-1.98)	0.000	19.8		
Step 1	98	1.44 (1.16-1.79)	0.001	11.1		
Step 2	98	1.39 (1.12-1.73)	0.003	8.96		
Step 3	77	1.27 (0.98-1.64)	0.073	3.21		
Sudden death					0.0027 (0.021)	0.08 (0.44)
Unadjusted	181	1.27 (1.09-1.47)	0.002	9.64		
Step 1	181	1.19 (1.02-1.38)	0.026	4.96		
Step 2	180	1.15 (0.98-1.34)	0.078	3.11		
Step 3	141	1.00 (0.84-1.20)	0.990	0.00		
Coronary end point					0.0011 (0.27)	0.05 (0.51)
Unadjusted	304	1.20 (1.07-1.34)	0.002	9.85		
Step 1	304	1.15 (1.01-1.29)	0.019	5.46		
Step 2	301	1.13 (1.01-1.27)	0.040	4.22		
Step 3	233	1.05 (0.91-1.21)	0.492	0.47		
Hospitalization, any cause					0.0007 (0.016)	0.04 (0.53)
Unadjusted	758	1.17 (1.09-1.26)	0.000	18.2		
Step 1	757	1.11 (1.03-1.19)	0.006	7.69		
Step 2	752	1.09 (1.01-1.17)	0.021	5.30		
Step 3	609	1.00 (0.92-1.09)	0.958	0.00		
Hospitalization, CV cause					0.0005 (0.56)	0.09 (0.34)
Unadjusted	568	1.18 (1.09-1.28)	0.000	15.6		

Step 1	567	1.14 (1.05-1.24)	0.002	9.67		
Step 2	564	1.13 (1.03-1.23)	0.006	7.53		
Step 3	463	1.03 (0.94-1.14)	0.492	0.47		
Hospitalization from WHF					0.0002 (0.75)	0.06 (0.47)
Unadjusted	310	1.30 (1.16-1.46)	0.000	20.4		
Step 1	309	1.24 (1.11-1.39)	0.000	13.7		
Step 2	308	1.21 (1.08-1.36)	0.001	10.6		
Step 3	255	1.04 (0.91-1.19)	0.559	0.34		

YKL-40, log transformed per SD, as predictor of outcome. All Hazard Ratios (HR) are given as HR (95% confidence interval). C index, Δ; difference in C index between fully adjusted model with and without inclusion of YKL-40, corresponding (*p*-value). Net Reclassification Improvement (NRI); calculated from C-indexes for fully adjusted models with and without inclusion of YKL-40, corresponding (*p*-value). Unadjusted (n=1344). The models are adjusted as follows: Step 1 (n=1342): Ejection fraction, New York Heart Association functional class, age, body mass index, diabetes mellitus, sex, intermittent claudication and heart rate. Step 2 (n=1333): All variables from Step 1 as well as ApoB/Apo A-1 ratio and estimated glomerular filtration rate. Step 3 (1111): all variables from Step 2 as well as C-reactive protein and amino-terminal pro B-type natriuretic peptide. CV, cardiovascular; WHF, worsening heart failure.

Figure legends

Figure 1

Kaplan-Meier curves for the primary end point, coronary endpoint, all-cause- and CV mortality according to tertile of YKL-40 levels. T1, T2 and T3, represents tertile 1 to 3.

Figure 2

Interactions between treatment and serum levels of YKL-40 at baseline and association with outcomes in the CORONA trial. For each tertile (T1, T2 and T3) the fully adjusted HR and 95% CI is plotted. The p-values indicate the interaction by treatment term for each outcome (i.e. log-transformed standardized YKL-40*treatment).

Figure 3

Change in total cholesterol according to treatment and YKL-40 tertile.

Figure 4

Comparison with a previous study in HF. **A.** restricted cubic spline analysis of baseline YKL-40 showing tertile (T1, T2 and T3) limits. **B.** Correlation between serum YKL-40 level using commercial enzyme immunoassay (EIA) from QUIDEL and R&D. **C.** Comparison of serum YKL-40 levels in our study divided into quartiles after normalizing the differences between the two EIA's and the study by Harutyunyan et al.(14) **D.** Kaplan-Meier curves for the primary endpoint, coronary endpoint, all-cause- and CV mortality according to quartiles of YKL-40.