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CLINICAL RESEARCH
Coronary heart disease

High serum YKL-40 concentration is associated with cardiovascular and all-cause mortality in patients with stable coronary artery disease

Jens Kastrup[1]*†, Julia S. Johansen[2†], Per Winkel[3], Jørgen Fischer Hansen[4], Per Hildebrandt[5], Gorm Boje Jensen[6], Christian M. Jespersen[4], Erik Kjøller[7], Hans Jørn Kolmos[8], Inga Lind[9], Henrik Nielsen[10], Christian Gluud[3], and the CLARICOR Trial Group

[1]Department of Medicine B, Cardiac Catheterization Laboratory 2014, The Heart Centre, Rigshospitalet, Copenhagen University Hospital and Faculty of Health Sciences, Blegdamsvej 9, DK-2100 Copenhagen, Denmark; [2]Department of Rheumatology, Copenhagen University Hospital, Copenhagen, Denmark; [3]The Copenhagen Trial Unit, Centre for Clinical Intervention Research, Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark; [4]Department of Cardiology, Bispebjerg Hospital, Copenhagen University Hospital, Copenhagen, Denmark; [5]Department of Cardiology, Frederiksberg Hospital, Copenhagen University Hospital, Copenhagen, Denmark; [6]Department of Cardiology, Hvidovre Hospital, Copenhagen University Hospital, Copenhagen, Denmark; [7]Department of Cardiology, Herlev Hospital, Copenhagen University Hospital, Copenhagen, Denmark; [8]Department of Clinical Microbiology, Odense University Hospital, Denmark; [9]Statens Serum Institute, Copenhagen, Denmark; and [10]Department of Cardiology, Amager Hospital, Copenhagen University Hospital, Copenhagen, Denmark

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Aims Macrophages in atherosclerotic plaques secrete YKL-40. We tested the hypothesis if high serum YKL-40 concentration predicts coronary events and death of patients with stable coronary artery disease (CAD).

Methods and results During the 2.6 years follow-up period (median 2.77 year, interquartile range 0.23 year), 270 patients among the 4298 patients with stable CAD in the CLARICOR trial suffered myocardial infarction (MI) and 377 died (187 classified as cardiovascular death). Serum YKL-40 transformed as Y=log[max(82, serum YKL-40/μg/L)] was significantly associated with cardiovascular death [hazard ratio (HR) = 1.88, 95% confidence interval (CI) = 1.54–2.31, $P < 0.001$], all-cause mortality (HR = 2.01, 95% CI = 1.75–2.31, $P < 0.001$), and MI (HR = 1.38, 95% CI = 1.13–1.68, $P = 0.002$). Following multivariable adjustment for cardiovascular risk factors (age, sex, previous MI, smoking status, hypertension, diabetes mellitus) and selected medical treatments Y contributed significantly to prediction of all-cause mortality ($P < 0.001$) and cardiovascular mortality ($P = 0.001$), but not MI ($P = 0.25$).

Conclusion High serum YKL-40 is associated with MI, cardiovascular and all-cause mortality in patients with stable CAD.

Keywords Biomarker • Coronary artery disease • Heart disease • Prognostic factors • YKL-40

Introduction

Circulating levels of YKL-40 is a potential biomarker of acute and chronic inflammation and tissue remodelling,[1–7] including systemic low-grade inflammation[8] and YKL-40 may participate in the innate immune response.[2] YKL-40 is regarded as an acute phase protein, since its plasma concentration is increased in inflammatory diseases.[1–5] YKL-40 is a phylogenetically highly conserved chitin-, heparin-, and collagen-binding protein with homologues in vertebrates and invertebrates, and is a member of 'mammalian chitinase-like proteins'.[3,9]

The full biological functions of YKL-40 are unknown. YKL-40 is a growth factor for fibroblasts[10] and has an anti-catabolic effect preserving extracellular matrix during tissue remodelling.[11] YKL-40 stimulates migration and adhesion of endothelial cells and vascular smooth muscle cells (VSMCs), suggesting a role in angiogenesis,[12–14] and may play a role in regulating response of cancer cells to hypoxia.[15] Atherosclerotic plaque macrophages express

* Corresponding author. Tel: +45 35 45 28 19/28 17, Fax: +45 35 45 27 05, Email: jens.kastrup@rh.regionh.dk

†These authors contributed equally to this article.

YKL-40, particularly macrophages that have infiltrated deeper into the lesion, and the highest YKL-40 mRNA expression is found in macrophages in the early atherosclerotic lesion.[16]

Studies have suggested that high serum YKL-40 levels could be a prognostic biomarker of short survival. This is demonstrated in 80-year-old people[8] and in patients with *Streptococcus pneumoniae* bacteraemia,[1] and local or metastatic cancer.[7] Serum YKL-40 levels are elevated in patients with acute myocardial infarction (MI)[17,18] and stable coronary artery disease (CAD),[19] and associated with the number of diseased vessels assessed by coronary angiography.[19]

In the present study, we tested the hypothesis that elevated serum YKL-40 is a risk factor for acute coronary syndrome and death in patients with stable CAD.

Methods

Patients

The patients were included in the randomized, placebo-controlled, multi-centre CLARICOR trial of patients with stable CAD treated for 2 weeks with oral clarithromycin 500 mg once daily (Klacid Uno®, Abbott, UK) or a matching placebo.[20] The patients had a diagnosis of MI or angina pectoris (AP) (ICD codes 209–219), percutaneous transluminal coronary angioplasty, or coronary bypass surgery during the years 1993–99. Exclusion criteria included MI or unstable AP within the previous 3 months, percutaneous transluminal coronary angioplasty or coronary bypass surgery within the previous 6 months, New York Heart Association class IV cardiac failure, impaired renal or hepatic function, or cancer. In this study, 4373 patients were randomized between 5 October 1999 and 15 April 2000; 4350 patients donated blood and serum was available for YKL-40 determination in 4298 patients.

Follow-up

The patients had a 2.6 years follow-up period (median 2.77 years, interquartile range 0.23 year). Information about fatal and non-fatal admissions came from the Danish National Hospital Register, a database of all somatic hospital admissions. Information about death came from the Danish Central Civil Register, which records the vital status of all inhabitants in Denmark. Registration is 100% in these registers. On the basis of these registers, the coordinating centre collected death certificates and copies of hospital records during the follow-up period and forwarded each potential event separately to the event committee. As the observed event rate was lower than estimated, the steering committee extended the follow-up period from 2 to 3 years without analysing the data. Final data were collected April 2003.[20]

Outcomes

In the present study, we examined time from randomization to re-admission for: (1) unstable AP, (2) MI, (3) cardiovascular death, and (4) any death. We used pre-specified outcome flowchart forms to search for possible outcomes. Two randomly chosen members of the event committee, which consisted of three cardiologists, evaluated copies of hospital records and death certificates. In case of disagreement between the two evaluations, both forms together with copies of the record of the event were given to the third member, who had to select the most likely option. In cases with inadequate information, we classified the cause of death as unknown. If sufficient information was available, we judged death to be due to cardiovascular disease unless a non-cardiovascular cause was present. In the flowchart, the evaluation was performed in concordance with the international form of medical certificate of cause of death after The Manual of the International Statistical Classification of Diseases, Injuries and Causes of Death (World Health Organisation. Manual of the international statistical classification of diseases, injuries and causes of death. 9th revision. Geneva: WHO, 1974). The potential classification codes (and the corresponding ICD10 codes) were: acute MI (I21), revascularization due to AP (I20), heart failure (I50), cerebrovascular disease (I63), and other cardiovascular disease (i.e. I10–I79 except for the previous mentioned).

Long-lasting chest pain or chest pain at rest without major changes in cardiac enzymes (creatine kinase-isoenzyme MB or troponin) was classified as unstable AP. An elevation of cardiac enzymes and significant ST changes in the electrocardiogram consistent with myocardial ischaemia or MI were classified as MI.[20]

Ethics

The trial was approved by the regional Ethics Committee (KF 01-076/99), the Danish Medicines Agency (2612-975), and the Danish Data Protection Agency (199-1200-174), registered at *ClinicalTrials.gov* (NCT00121550) and conducted according to the Declaration of Helsinki. Participants gave written informed consent.

YKL-40 analysis

Serum concentrations of YKL-40 were determined in duplicates by a commercial ELISA (Quidel, San Diego, CA, USA) using streptavidin-coated microplate wells, a biotinylated-Fab monoclonal capture antibody, and an alkaline phosphatase-labelled polyclonal detection antibody. The recovery is 102%, detection limit 20 μg/L, intra-assay coefficient of variation (CV) $< 5.0\%$, and inter-assay CVs are $<5.4\%$ (low control) and $<6.7\%$ (high control) in the 127 ELISA kits used for the analysis of the 4298 samples.

Statistical analysis

Cox-analyses were used to analyse the effect of one or more covariates on the time to an event. Extended Cox analyses including a covariate, time, and the interaction between time and the covariate as variables was used to test the proportionality assumption of the Cox model. The latter test was supplemented by a visual assessment of the log–log plots of the groups of the covariate. The assumption of the Cox model of linearity between a continuous covariate and the logarithm of the hazard ratio (HR) was assessed from an inspection of the relationship between the log HR and the mean value of the groups defined by the deciles of the covariate's distribution.

The proportionality assumption of the Cox model was fulfilled for all four events. The assumption for the Cox model of linearity between serum YKL-40 and the logarithm of the HR was not fulfilled in any of the analyses. Since there was no relationship between serum YKL-40 and the log of the HR of any of the events at serum YKL-40 levels below 82 μg/L and a linear relationship between the log of YKL40 and log HR above this value, we transformed serum YKL-40 using the transformation: $Y=\log[\max(82, \text{serum YKL-40}/\mu g/L)]$. Age fulfilled the linearity assumption in case of the events all-cause death and cardiac death. For MI the transformation $Y=\max(63, \text{age/year})$ was used.

If transformed serum YKL-40 had a significant effect on the time to an event, we repeated the analysis adjusting for the known risk factors (age, sex, previous MI, smoking status, hypertension, diabetes mellitus).

If the effect was still significant, we included selected indicators of treatment in the analysis. These factors were selected as follows: A random sample comprising one-half of the patients was selected and an analysis of the corresponding data material including as covariates the risk factors and all indicators of medical treatment (beta-blocker, ACE-inhibitor, calcium blocker, statin, magnyl, long-lasting nitrates, digoxin, diureticas, anti-arrhythmicas) was done. In this analysis, all covariates except the treatment indicators were forced to stay in the analysis which comprised a backward elimination using the likelihood ratio test with $P = 0.10$ for removal of variables. The analysis was repeated using the other half of the data material. The treatment indicators selected were those that were retained in both of these two analyses. In all analyses the intervention indicator was forced to be included in the model.

Since the distributions of serum YKL-40 as well as those of log (YKL-40) differed significantly from the Gaussian distribution as assessed by the Shapiro–Wilk *W* test, non-parametric tests (Mann–Whitney test) were used to compare serum YKL-40 levels between patient groups.

The level of significance used was 0.05 and all tests were two-sided. To account for the inflation of the experiment wise Type I error due to multiple testing, we used the Bonferroni correction giving a significance level of 0.05/22=0.0023 since 22 tests were done. The analyses were done using the SPSS version 15.0.

Results

Serum YKL-40 in relation to demographic and clinical characteristics of the patients with stable coronary artery disease

The demographic data and the relationship between serum concentrations of YKL-40 and the demographic data of the 4298 patients with stable CAD are described in *Table 1*. The median serum YKL-40 was 110 μg/L (range 20–3047 μg/L). Serum YKL-40 was higher in patients with age >60 years, diabetes, hypertension, and in patients treated with calcium-antagonist, ACE-inhibitors, long-lasting nitrates, diuretics, and digoxin compared with patient without (*Table 1*). In contrast, patients treated with statin and magnyl had lower serum YKL-40 compared with untreated patients (*Table 1*).

Table 1 Serum YKL-40 concentrations (μg/L, given as median and 25th and 75th percentiles) in the patients with stable coronary artery disease according to demographic, clinical, and medical characteristics

Quantities		*n*	Median	25 percentile	75 percentile	*P*-value of difference
Sex	Males	2985	111	75	169	0.77
	Females	1313	109	77	166	
Age	≥60 year	3016	119	82	180	<0.001
	<60 years	1282	92	64	135	
Diabetes	Yes	661	124	85	195	<0.001
	No	3637	108	75	163	
Hypertension	Yes	1731	116	79	179	<0.001
	No	2567	106	73	160	
Smoking habits	Smoker	1545	112	78	172	0.022
	Non-smoker	2753	107	74	166	
Previous MI	Yes	2914	111	77	170	0.04
	No	1384	106	73	163	
Magnyl	Yes	3778	109	75	166	0.003
	No	520	116	82	189	
Beta-blocker	Yes	1314	108	75	164	0.28
	No	2984	111	76	170	
Calcium antagonist	Yes	1503	113	78	177	0.009
	No	2795	108	74	164	
ACE-inhibitor	Yes	1159	118	78	177	0.001
	No	3139	107	75	164	
Long-lasting nitrates	Yes	902	121	85	189	<0.001
	No	3396	107	73	163	
Diuretics	Yes	1506	128	86	194	<0.001
	No	2792	102	73	86	
Digoxin	Yes	275	139	97	211	<0.001
	No	4023	108	75	166	
Statin	Yes	1768	102	72	151	<0.001
	No	2530	116	79	177	
Anti-arrhythmicas	Yes	101	123	84	203	0.11
	No	4197	110	75	167	

Serum YKL-40 as predictor of unstable angina pectoris, myocardial infarction, cardiovascular death, and all-cause mortality in patients with stable coronary artery disease

During the 2.6 years follow-up, a new unstable coronary syndrome event occurred in 373 patients (8.7%), including 120 patients with unstable AP (2.8%) and 270 with MI (6.3%). Seventeen patients were registered with both unstable AP and MI (0.4%). In the entire population a total of 384 died, 189 (49.2%) had a cardiovascular death, 77 (20.1%) died due to cancer, 84 (21.9%) died from other causes (e.g. pneumonia, sepsis, chronic obstructive pulmonary disease, and asthma), and 34 (8.9%) died from unknown cause. One hundred and thirty-three patients died in hospital and 251 died before being admitted to hospital. In the YKL-40 study population 187 patients suffered cardiovascular death (4.4%) and a total of 377 patients died (8.8%).

Y (the transformed serum YKL-40) was significantly associated with cardiovascular death [HR = 1.88, 95% confidence interval (CI) = 1.54–2.31, $P < 0.001$], all-cause mortality (HR = 2.01, 95% CI = 1.75–2.31, $P < 0.001$), and MI (HR = 1.38, 95% CI = 1.13–1.68, $P = 0.002$), but not unstable AP ($P = 0.85$) (*Table 2*). Following multivariable adjustment for cardiovascular risk factors (age, sex, previous MI, smoking status, hypertension, diabetes mellitus) Y contributed significantly to the prediction of all-cause mortality (HR = 1.67, 95% CI = 1.43–1.95, $P < 0.001$) and cardiovascular mortality (HR = 1.51 95% CI = 1.20–1.89, $P = 0.001$), but not MI ($P = 0.26$) (*Table 2*). In the analysis where the predictive significance of medical treatments was assessed while all risk factors were forced to remain in the analysis (see section on Statistical analysis), treatment with diuretics, digoxin, and statin were retained in the analysis of time to death. In the analysis of time to cardiovascular death, only treatment with digoxin was retained, and in the analysis of time to MI only ACE-inhibitor was retained. Following multivariable adjustment for cardiovascular risk factors and the selected medical treatment indicators Y contributed significantly to prediction of all-cause mortality (HR = 1.62, 95% CI = 1.37–1.90, $P < 0.001$) and cardiovascular mortality (HR = 1.52, 95% CI = 1.20–1.92, $P = 0.001$) (*Table 2*).

For illustrative purposes, we show the event-free survival for unstable AP, MI, cardiovascular death, and all-cause mortality during the 2.6 years of follow-up for six serum YKL-40 groups in *Figure 1A–D*.

Discussion

We observed that high serum YKL-40 is a significant predictor for cardiovascular death, all-cause mortality, and MI in patients with stable CAD. Correcting the analyses for risk factors and selected medical treatments had a moderate impact on the HRs for all-cause mortality and cardiovascular death, but eliminated the relation to MI. High serum YKL-40 was not associated with unstable AP, however the number of these patients was small.

The strengths of the present study are the size of the patient population, no losses to follow-up of events, and the ethnic homogeneity of the patient population. A weakness is that only serum YKL-40 was determined and not other potential prognostic biomarkers of MI and death in patients with CAD, e.g. C-reactive protein and B-type natriuretic peptide (BNP).[21–26] In patients with stable CAD, the highest serum NT-pro-BNP level had an HR of 2.4 for all-cause mortality,[22] and the highest serum CRP level had an HR for cardiac death of 1.75, and for all-cause mortality of 1.78 during a 4.8 years follow-up period.[23] In the WIZARD trial including 3319 patients with stable CAD treated with azithromycin or placebo using serum CRP as a biomarker, the HR for all-cause mortality was 2.18 in the 3.1 years follow-up period.[24] In the present study, with a shorter follow-up (2.6 years), the HRs using serum YKL-40 as a biomarker were 1.38 for MI, 1.88 for cardiovascular death, and 2.01 for all-cause mortality. Due to differences in study populations, a direct comparison of HRs between different biomarkers in published studies is not valid.

Table 2 The effect of f(YKL-40)[a] on time to death, to cardiovascular death, and myocardial infarction alone or in combination with risk factors and risk factors plus selected indicators of treatment

Event	Co-variates included in Cox analysis								
	f(YKL-40) + intervention indicator[b]			f(YKL-40) + intervention indicator + risk factors[c]			f(YKL-40) + intervention indicator + risk factors + selected treatment indicators[d]		
	HR	95% CI	*P*-value	HR	95% CI	*P*-value	HR	95% CI	*P*-value
Death	2.01	1.75–2.31	<0.001	1.67	1.43–1.95	<0.001	1.62	1.37–1.90	<0.001
Cardiovascular death	1.88	1.54–2.31	<0.001	1.51	1.20–1.89	<0.001	1.52	1.20–1.92	0.001
Myocardial infarction	1.38	1.13–1.68	0.002	1.13	0.91–1.41	0.26	1.14	0.91–1.41	0.25

HR, hazard ratio; CI, confidence interval.
[a]f(YKL-40)=log[max(82, serum YKL-40/μg/L)].
[b]Indicator of treatment with clarithromycin included *a priori* in all analyses (reference: placebo).
[c]Age, sex, hypertension, diabetes, smoking habits, and previous myocardial infarction.
[d]Treatment indicators for diuretics, digoxin, and statins were included in the analysis of death. In the analysis of cardiovascular death only the indicator of digoxin treatment was included. In the analysis of myocardial infarction only that of ACE-inhibitor treatment was included.

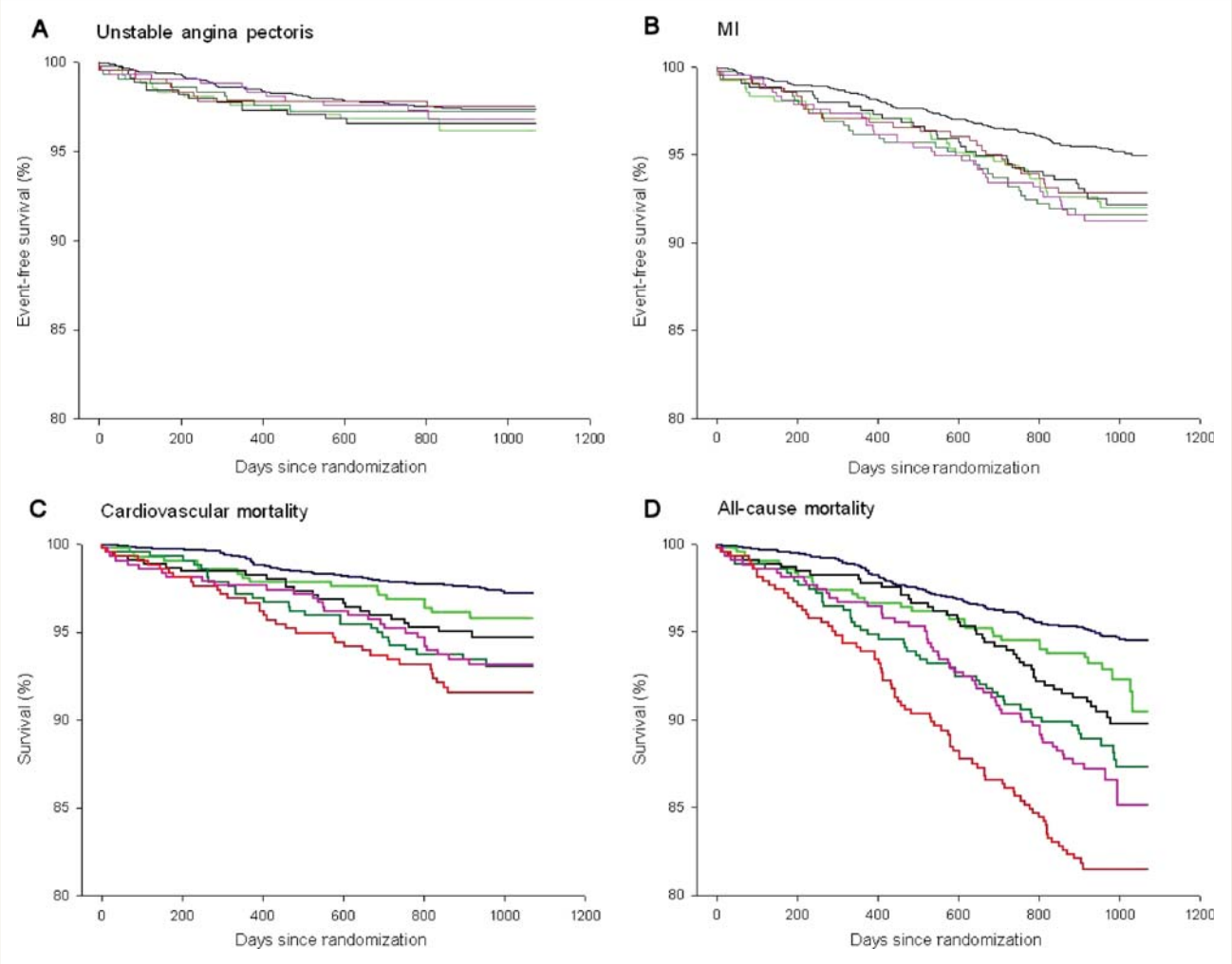

Figure 1 Event-free survival for (*A*) unstable angina pectoris, (*B*) myocardial infarction (MI), (*C*) cardiovascular death, and (*D*) all-cause mortality during 2.6 years of follow-up of each of six serum YKL-40 groups. YKL-40 $<$ 110 μg/L (blue), 110 μg/L $\leq$ YKL-40 $<$ 129 μg/L (green), 129 μg/L $\leq$ YKL-40 $<$ 153 μg/L (black), 153 μg/L $\leq$ YKL-40 $<$ 191 μg/L (dark green), 191 μg/L $\leq$ YKL-40 $<$ 256 μg/L (pink), and 256 μg/L $\leq$ YKL-40 (red). These groups were formed by first dividing the serum YKL-40 levels into 10 groups using the deciles of the serum YKL-40 distribution. Then the first five of these groups were combined into one to obtain in all the above six groups.

Since risk factors such as BMI, renal status, as well as other potential biomarkers were not measured in the present study the significance of serum YKL-40 in the prediction of all-cause death combined with those risk factors examined in the study by Pencina *et al.*[27] cannot be meaningfully assessed. Future studies with all relevant risk factors included should evaluate whether serum YKL-40 provide substantially better prediction of all-cause mortality in patients with stable CAD than that obtained using all known and relevant risk factors alone.

Our results suggest that serum YKL-40 could be a new biomarker of acute and chronic inflammation in patients with stable CAD. Circulating YKL-40 may reflect the total burden of coronary atherosclerosis or identify a high-risk atherosclerosis phenotype with ongoing inflammation and atherosclerotic plaque formation. The molecular processes governing the induction of YKL-40 and its precise biological functions are unknown. In contrast to CRP, mainly produced by hepatocytes in response to high IL-6, YKL-40 is produced by macrophages and neutrophils in tissues with inflammation[2,3,5,16,28] and by differentiated macrophages and activated neutrophils.[29,30] Interestingly, macrophages in atherosclerotic plaques express YKL-40 mRNA, particularly macrophages located deep in the lesion, and the highest YKL-40 expression is found in macrophages in the early lesion of atherosclerosis.[16] YKL-40 is also synthesized by VSMCs and promotes their migration and attachment,[12–14] suggesting a role in the process of atherosclerotic plaque formation, where VSMCs are induced to migrate through the intima in response to exogenous signals. *In vitro* YKL-40 is synthesized by swine VSMCs isolated from the thoracic aorta during the time of transition from a proliferating monolayer culture to a non-proliferating differentiated multilayer culture, and YKL-40 secretion continued as VSMCs reorganize and differentiate.[12–14] This nodule forming process mimics some of the characteristics of the *in vivo* changes that occur in VSMCs following injury, where media SMCs dedifferentiate, migrate, and

contribute to the process of vascular restenosis and neointima formation. Furthermore, YKL-40 modulates vascular endothelial cell morphology by promoting the formation of branching tubules, indicating a role in angiogenesis by stimulating the migration and reorganization of vascular endothelial cells.[13,14]

However, some of the circulating YKL-40 in our group of patients with CAD who died of cancer during follow-up, may have originated from cancer cells.[7] Since YKL-40 is not disease specific co-morbidity should always be considered, and it has recently been found that elevated plasma YKL-40 predicted increased risk of gastrointestinal cancer in the general population.[31]

Serum YKL-40 is increased up to seven-fold in both patients with stable CAD and after an acute ST-elevation MI (STEMI)[17–19] and associated with the number of angiographically demonstrated coronary artery stenosis.[19] This supports the hypothesis, that YKL-40 plays a role in both the acute inflammatory process eliciting or responding to protect against the plaque instability and in the recovery and remodelling process after an acute STEMI by promoting the growth of new cardiomyocytes and inducing angiogenesis. YKL-40 may protect cardiomyocytes from undergoing apoptosis under ischaemia, since YKL-40 expression is up-regulated in cancer cells following hypoxia.[32] It has to be demonstrated in future studies, whether serum YKL-40 will fulfil the three fundamental requirements to be a potentially useful new biomarker: (1) analytical methods that allow reliable measurement are available to every clinician; (2) provide prognostic information in multiple studies and diverse populations, with incremental prognostic information when added to validated risk scores; and (3) help the clinician to manage patients.[33] Only requirement (1) is fulfilled at present, since we used a commercially available YKL-40 ELISA. This method takes less than 4 h, has low intra- and inter-assay coefficients of variation, and the intraclass correlation coefficient indicated reasonable reliability of serum YKL-40 measurements and relatively small variation in healthy subjects.[34]

In conclusion, serum YKL-40 was found to be a biomarker for MI, cardiovascular death, and all-cause mortality in patients with stable CAD. The mechanism(s) involved cannot be resolved from the present study and has to be clarified in future studies. We speculate if serum YKL-40 could be used for monitoring the sufficiency of medical treatment of patients with stable CAD and thereby assist in the reduction of the high occurrence of non-fatal and fatal cardiovascular events in these patients.

Contributors

J.K. was the inventor and coordinator of the YKL-40 study. J.K., J.F.H., P.H., G.B.J., C.M.J., E.K., H.J.K., I.L., H.N., and C.G. are members of the CLARICOR Trial group. C.G. and C.M.J. coordinated the CLARICOR Trial. P.W. and C.G. are guarantors and have full access to all data in the study and take responsibility for the integrity of data and accuracy of the data analysis. E.K. was chairman of the Event Committee. J.V. Petersen and P. Hughes (deceased) coordinated the trial data accrual and data entry. B. Hansen, N. Frydendahl, B. Hødholdt, K. Juliussen, and M. Hansen executed data accrual. K. Nillson and N. Salas developed the randomization system and the data management systems. Statistical expertise: P.W., C.G. YKL-40 measurements: J.S.J. Analysis and interpretation of data: All authors. Drafting of the manuscript: J.K., J.S.J., P.W., and C.G. Critical revision of the manuscript for important intellectual content: All authors. Obtained funding for the YKL-40 study: J.K. and J.S.J. All authors have participated in the reporting stage of this manuscript, and have seen and approved the final version.

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Conflict of interest: A European patent (No. PA 2008 00089 'Classification of individuals suffering from cardiovascular diseases according to survival prognoses as found by measuring the levels of biomarker YKL-40') was issued on 23 January 2008, and is exclusively licensed to J.K. The authors have no conflicting financial interests except for P.H., who received consulting fees from Astra Zenica, Merck, Sharpe and Dohme, sanofi-aventis, and Servier, lecture fees from Roche Diagnostics, Astra Zenica, and Servier, and a research grant from Roche Diagnostics.

References

1. Kronborg G, Østergaard C, Weis N, Nielsen H, Obel N, Pedersen SS, Price PA, Johansen JS. Serum level of YKL-40 is elevated in patients with *Streptococcus pneumoniae* bacteremia and is associated to the outcome of the disease. *Scand J Infect Dis* 2002;**34**:323–326.
2. Chupp GL, Lee CG, Jarjour N, Shim YM, Holm CT, He S, Dziura JD, Reed J, Coyle AJ, Kiener P, Cullen M, Grandsaigne M, Dombret M-C, Aubier M, Pretolani M, Elias JS. A chitinase-like protein in the lung and circulation of patients with severe asthma. *N Engl J Med* 2007;**357**:2016–2027.
3. Johansen JS. Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodelling, fibrosis and cancer. *Dan Med Bull* 2006;**53**:172–209.
4. Rathcke CN, Johansen JS, Vestergaard H. YKL-40, a biomarker of inflammation, is elevated in patients with type 2 diabetes and is related to insulin resistance. *Inflamm Res* 2006;**55**:53–59.

5. Johansen JS, Baslund B, Garbarsch C, Hansen M, Stoltenberg M, Lorenzen I, Price PA. YKL-40 in giant cells and macrophages from patients with giant cell arthritis. *Arthritis Rheum* 1999;**42**:2624–2630.
6. Nøjgaard C, Johansen JS, Christensen E, Skovgaard LT, Price PA, Becker U, and The EMALD Group. Serum levels of YKL-40 and PIIINP as prognostic markers in patients with alcoholic liver disease. *J Hepatol* 2003;**39**:179–186.
7. Johansen JS, Jensen BV, Roslind A, Nielsen D, Price PA. Serum YKL-40, a new prognostic biomarker in cancer patients? Review. *Cancer Epidemiol Biomarkers Prev* 2006;**15**:194–202.
8. Johansen JS, Pedersen AN, Schroll M, Jørgensen T, Pedersen BK, Bruunsgaard H. High serum YKL-40 level in a cohort of octogenarians is associated with increased risk of all-cause mortality. *Clin Exp Immunol* 2008;**151**:260–266.
9. Bussink AP, Speijer D, Aerts JMFG, Boot RG. Evolution of mammalian chitinase(-like) members of family 18 glycosyl hydrolases. *Genetics* 2007;**177**:959–970.
10. Recklies AD, White C, Ling H. The chitinase 3-like protein human cartilage 39 (HC-gp39) stimulates proliferation of human connective-tissue cells and activates both extracellular signal-regulated kinase- and protein kinase B-mediated signalling pathways. *Biochem J* 2002;**365**:119–126.
11. Ling H, Recklies AD. The chitinase 3-like protein human cartilage glycoprotein 39 inhibits cellular responses to the inflammatory cytokines interleukin-1 and tumour necrosis factor-alpha. *Biochem J* 2004;**380**:651–659.
12. Shackelton LM, Mann DM, Millis AJT. Identification of a 38-kDa heparin-binding glycoprotein (gp38 k) in differentiating vascular smooth muscle cells as a member of a group of proteins associated with tissue remodeling. *J Biol Chem* 1995;**270**:13076–13083.
13. Malinda KM, Ponce L, Kleinman HK, Shackelton LM, Millis AJT. Gp38 k, a protein synthesized by vascular smooth muscle cells, stimulates directional migration of human umbilical vein endothelial cells. *Exp Cell Res* 1999;**250**:168–173.
14. Nishikawa KC, Millis AJT. Gp38 k (CHI3L1) is a novel adhesion and migration factor for vascular cells. *Exp Cell Res* 2003;**287**:79–87.
15. Saidi A, Javerzat S, Bellahcene A, de Vos J, Bello L, Castronovo V, Deprez M, Loiseau H, Bikfalvi A, Hagedorn M. Experimental anti-angiogenesis causes upregulation of genes associated with poor survival in glioblastoma. *Int J Cancer* 2008;**122**:2187–2198.
16. Boot RG, van Achterberg TAE, van Aken BE, Renkema GH, Jacobs MJHM, Aerts JMFG, de Vries CJM. Strong induction of members of the chitinase family of proteins in atherosclerosis. Chitotriosidase and human cartilage gp-39 expressed in lesion macrophages. *Arterioscler Thromb Vasc Biol* 1999;**19**:687–694.
17. Nøjgaard C, Høst NB, Christensen IJ, Poulsen SH, Egstrup K, Price PA, Johansen JS. Serum levels of YKL-40 increases in patients with acute myocardial infarction. *Coron Artery Dis* 2008;**19**:257–263.
18. Wang Y, Ripa SR, Johansen JS, Gabrielsen A, Steinbrüchel D, Friif T, Bindslev L, Haack-Sørensen M, Jørgensen E, Kastrup J. YKL-40 a new biomarker in patients with acute coronary syndrome or stable coronary artery disease. *Scand Cardiovasc J* 2008;**42**:295–302.
19. Kucur M, Isman FK, Karadag B, Vural VA. Tavsanoglu S. Serum YKL-40 levels in patients with coronary artery disease. *Coron Artery Dis* 2007;**18**:391–396.
20. Jespersen CM, Als-Nielsen B, Damgaard M, Hansen JF, Hansen S, Helø OH, Hildebrandt P, Hilden J, Jensen GB, Kastrup J, Kolmos HJ, Kjøller E, Lind I, Nielsen H, Petersen L, Gluud C, the CLARICOR Trial Group. Randomised placebo controlled multicentre trial to assess short term clarithromycin for patients with stable coronary heart disease: CLARICOR trial. *BMJ* 2006;**332**:22–27.
21. Willerson JT, Ridker PM. Inflammation as a cardiovascular risk factor. *Circulation* 2004;**109**(Suppl. II):II-2–II-10.
22. Kragelund C, Grønning B, Køber L, Hildebrandt P, Steffensen R. N-terminal pro-B-type natriuretic peptide and long-term mortality in stable coronary artery disease. *N Engl J Med* 2005;**352**:666–675.
23. Sabatine MS, Morrow DA, Jablonski KA, Rice MM, Warnica JW, Domanski MJ, Hsia J, Gersh BJ, Rifai N, Ridker PM, Pfeffer MA, Braunwald E. Prognostic significance of the centers for disease control/American Hearth Association high-sensitivity C-reactive protein cut points for cardiovascular and other outcomes in patients with stable coronary artery disease. *Circulation* 2007;**115**:1528–1536.
24. Aguilar D, Fisher MR, O'Connor CM, Dunne MW, Muhlestein JB, Yao L, Gupta S, Benner RJ, Cook TD, Edwards D, Pfeffer MA. Metabolic syndrome, C-reactive protein, and prognosis in patients with established coronary artery disease. *Am Heart J* 2006;**152**:298–304.
25. Shlipak MG, Ix JH, Bibbins-Domingo K, Lin F, Whooley MA. Biomarkers to predict recurrent cardiovascular disease: the heart and soul study. *Am J Med* 2008;**121**:50–57.
26. Zethelius B, Berglund L, Sundström J, Ingelsson E, Basu S, Larsson A, Venge P, Ärnlöv J. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. *N Engl J Med* 2008;**358**:2107–2116.
27. Pencina MJ, D'Agostino RB Sr, D'Agostino RB Jr, Vasan RS. Evaluating the added predictive ability of a new marker: from area under the ROC curve to reclassification and beyond. *Statist Med* 2008;**27**:157–172.
28. Junker N, Johansen JS, Andersen CB, Kristjansen PEG. Expression of YKL-40 by peritumoral macrophages in human small cell lung cancer. *Lung Cancer* 2005;**48**:223–231.
29. Rehli M, Niller H-H, Ammon C, Langmann S, Schwarzfischer L, Andreesen R, Krause SW. Transcriptional regulation of CHI3L1, a marker gene for late stages of macrophage differentiation. *J Biol Chem* 2003;**278**:44058–44067.
30. Volck B, Price PA, Johansen JS, Sørensen O, Benfield T, Calafat J, Nielsen HJ, Borregaard N. YKL-40, a mammalian member of the chitinase family, is a matrix protein of specific granules in human neutrophils. *Proc Assoc Am Phys* 1998;**110**:351–360.
31. Johansen JS, Bojesen SE, Mylin AK, Frikke-Schmidt R, Price PA, Nordestgaard BG. Elevated plasma YKL-40 predicts increased risk of gastrointestinal cancer and decreased survival after any cancer diagnosis in the general population. *J Clin Oncol* 2008; Published online ahead of print December 15.
32. Junker N, Johansen JS, Hansen LT, Lund EL, Kristjansen PEG. Regulation of YKL-40 expression during genotoxic or microenvironmental stress in human glioblastoma cells. *Cancer Sci* 2005;**96**:183–190.
33. Morrow DA, de Lemos JA. Benchmarks for the assessment of novel cardiovascular biomarkers. *Circulation* 2007;**115**:949–952.
34. Johansen JS, Lottenburger T, Nielsen HJ, Jensen JEB, Svendsen MN, Kollerup G, Christensen IJ. Diurnal, weekly, and long-time variation in serum concentrations of YKL-40 in healthy subjects. *Cancer Epidemiol Biomarkers Prev* 2008;**17**:2603–2608.