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Prognostic value of 12 novel cardiological biomarkers in stable coronary artery disease. A 10-year follow-up of the placebo group of the Copenhagen CLARICOR trial

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ABSTRACT

Objective To assess if 12 novel circulating biomarkers, when added to 'standard predictors' available in general practice, could improve the 10-year prediction of cardiovascular events and mortality in patients with stable coronary heart disease.

Design The patients participated as placebo receiving patients in the randomised clarithromycin for patients with stable coronary artery disease (CLARICOR) trial at a random time in their disease trajectory.

Setting Five Copenhagen University cardiology departments and a coordinating centre.

Participants 1998 participants with stable coronary artery disease.

Outcomes Death and composite of myocardial infarction, unstable angina pectoris, cerebrovascular disease and death.

Results When only 'standard predictors' were included, 83.4% of all-cause death predictions and 68.4% of composite outcome predictions were correct. Log(calprotectin) and log(cathepsin-S) were not associated ($p \geq 0.01$) with the outcomes, not even as single predictors. Adding the remaining 10 biomarkers (high-sensitive assay cardiac troponin T; neutrophil gelatinase-associated lipocalin; osteoprotegerin; N-terminal pro-B-type natriuretic peptide; tumour necrosis factor receptor 1 and 2; pregnancy-associated plasma protein A; endostatin; YKL40; cathepsin-B), which were all individually significantly associated with the prediction of the two outcomes, increased the figures to 84.7% and 69.7%.

Conclusion When 'standard predictors' routinely available in general practices are used for risk assessment in consecutively sampled patients with stable coronary artery disease, the addition of 10 novel biomarkers to the prediction model improved the correct prediction of all-cause death and the composite outcome by <1.5%.

Trial registration number NCT00121550.

Strengths and limitations of this study

- Use of multiple biomarkers.
- Well-established cohort.
- Comprehensive statistical approach.
- Missing external validation.
- Relatively old cohort.

INTRODUCTION

Previously, we have studied the prognostic impact of routinely available 'standard predictors' when added to a prediction model void of covariates using the placebo receiving participants from the CLARICOR trial.[1–4] The impact, however, was quite modest.[1] For risk assessment of patients with coronary artery disease (CAD), there are a number of advanced biomarkers, including several from outside cardiology, which may help identifying CAD patients at high risk of cardiovascular (CV) disease manifestations.[2] Here, we assess the prognostic impact—relative to standard clinical predictors usually available during routine clinical work—of 12 newer biomarkers in predicting death and other serious CV events in patients suffering from CAD sampled while their disease was stable.

Briefly, the biomarkers are (1) serum N-terminal pro-B-type natriuretic peptide (pro-BNP), a marker of left ventricular dysfunction and heart failure; (2) high-sensitive assay cardiac troponin T (hs-cTnT) indicating myocardial ischaemia; (3) YKL40 found to be predictive of acute myocardial infarction (AMI), CV death and non-CV death; (4) the glycoprotein osteoprotegerin (OPG), which is positively related to coronary calcification,

vascular stiffness and the presence of unstable atherosclerotic plaques; (5) pregnancy-associated plasma protein A (PAPP-A), a marker of vulnerable plaques in coronary arteries; (6) cathepsin-B and (7) cathepsin-S, a group of proteinases that have been suggested to be causally involved in the different stages of the atherosclerotic process; (8) endostatin, an endogenous angiogenesis inhibitor suggested to mirror an increased neovascularisation induced by vascular or myocardial ischaemia; the soluble receptors, (9) soluble tumour necrosis factor receptor 1 (sTNFR1) and (10) soluble TNF receptor 2 (sTNFR2), suggested to portray information about a systemic inflammatory state that is independent of other more established inflammatory markers; (11) calprotectin and (12) neutrophil gelatinase-associated lipocalin (NGAL), both released from neutrophils when the cells are activated. Circulating levels of neutrophils and their activation products have been shown to be markers for plaque instability in both primary and secondary prevention of CV diseases.

All of these have been claimed to add some prognostic information in patients with stable coronary artery disease. Our group has tested the individual importance of many of these biomarkers, and in many studies statistical inference supports the view that biomarkers may improve the prediction.[5–12]

Our objectives were to clarify: (1) which of these newer biomarkers maintain their prognostic importance if all of them were simultaneously available and were combined with the routinely available clinical and laboratory information, and (2) what would then be their relative practical contribution if they were added to the 'standard predictors' such as age, smoking, plasma lipids, etc. In accordance with our published statistical analysis plan,[2] our analysis focusses on all-cause death and on a composite outcome comprising AMI, unstable angina pectoris (UAP), cerebrovascular disease (CeVD) and death.

MATERIALS

2 The patients

The study population is the placebo patients from the CLARICOR study.[3 4] Patients aged 18–85 years, from the Copenhagen area, who had a discharge diagnosis of myocardial infarction or angina pectoris during 1993–1999 and were alive in August 1999 were invited by letter for an interview and a 14-day trial of clarithromycin versus placebo.[3 4] Out of the 4372 who were randomised during October 1999 through April 2000, 2200 were in the placebo group.

The main results of the trial were that clarithromycin increased the risk of CV as well as all-cause death.[13–15] Therefore, we here focus on the placebo group.

For the CLARICOR trial, only patients who were in a stable state of their coronary heart disease were selected. Thus, patients were excluded if they fulfilled one or more of the following conditions: (1) had suffered from AMI or UAP within the previous 3 months; (2) had intracoronary interventions within the previous 6 months; (3) had impaired renal function; (4) had hepatic dysfunction; (5) had congestive heart failure (New York Heart Association IV classification of heart failure); (6) had active malignancy; (7) were without capacity to manage own affairs; (8) were breast feeding and (9) were possibly pregnant.

Of the 2200 participants one had garbled study data, and further 201 had one or more missing biomarker measurements (see below), leaving 1998 participants for the present analysis. Only 15 of these were lost track of due to emigration or disappearance.

2 The predictors

Information on smoking status, current medication, known hypertension, diabetes, sex, age and myocardial infarction at index hospitalisation or UAP was obtained from the local hospital files and patient interviews.

3 Biochemical measurements on serum collected at enrolment visit

Biochemical data were obtained from analysis of serum specimens sampled at inclusion of the patients and stored at - 80ºC. The quantities measured include lipoproteins,[16] high-sensitivity-C-reactive-protein/mg/L[7] and glomerular filtration rate/mL/min using creatinine.[17] These quantities together with the variables mentioned under predictors above are those collectively referred to as 'standard predictors'.

Biomarkers included as newer biomarkers were YKL40/µg/L[8]; hs-cTnT/ng/L[9]; binary pregnancy associated plasma protein-A (binary-PAPP-A), which is coded as 1 if PAPP-A was ≥4 mIU/L or 0 otherwise[10]; pro-BNP/ng/L[9]; cathepsin-B/µg/L[6 18]; endostatin/ng/mL[19]; cathepsin-S/µg/L[6 20]; sTNFR1/pg/mL and sTNFR2/pg/mL[5 21]; NGAL/ng/L[22]; calprotectin/mg/L[11] and OPG/ng/L.[12] Due to storage problems some marker data are missing on some patients.

2 The outcomes

Initial follow-up of the patients lasted for approximately 2.6 years, during which outcomes were collected through hospital and death registries and assessed by an adjudication committee.[4] Corresponding register data later produced similar results.[23 24] The adjudicated outcomes were therefore replaced and augmented by register outcomes to cover up to 10 years±3 months of follow-up. Last register follow-up was 31 December 2009. The public registers have an almost 100% coverage and the quality of these is described elsewhere.[25 26] The algorithm used to get from the International Statistical Classification of Diseases used in the national registries to the events of the composite outcome is described in detail previously.[13]

We assessed (1) the time from randomisation to all-cause death and (2) the time from randomisation until the first occurrence of one of the following outcomes: AMI, UAP, CeVD or all-cause death.

1 METHODS

2 Statistical analysis

The statistical principles and techniques used have previously been published.[1 2] While our previous publication[1] dealt with the prognostic impact of the 'standard predictors', we here use the same techniques to quantify the effect of adding biomarker information to the 'standard predictors'.

We used Cox regressions (SAS V.9.4), where all analyses that included covariates were stratified by centre. The assumption of proportional hazards over time covering all covariates included in a Cox analysis and the chosen functional form of quantitative covariates was tested using cumulative sums of martingale-based residuals over follow-up time and/or covariate values.[27]

We also analysed data using a parametric, accelerated failure-time model using the generalised gamma model of error.[28] A significance level of 0.01 was used to pinpoint empirical trends worthy of note. The logarithms of the present text are natural logarithms, so whenever the predictor is a log(serum concentration/unit), the HR is the factor by which the hazard increases when the logarithm increases by 1, that is, when the concentration increases by a factor e=2.72.

Biomarkers with an HR with p value ≥0.01 when used alone as covariate as well as when used in combination with the 'standard predictors' were excluded from further analyses. The remaining biomarkers were considered prognostic.

Assessment of the practical impact of using the set of newer biomarkers was obtained by comparing the per cent correct predictions obtained when the standard predictors were used alone with the percentage obtained when they were combined with the novel biomarkers using the method described earlier.[1]

Second, we report the areas under the receiver operating characteristics (ROCs), also known as area under the curves (AUCs) or *C*-indices, which one obtains when the Cox-Breslow risk estimates are matched against the events seen in the time window 0-to-9 years. The much-used binary (event vs no event) *C*-index is the concordance rate between risks and outcomes. It shows how frequently an event participant has a poorer prediction score than a non-event participant. In order to reward correct prediction of time of event, we further report Harrell's 'dynamic' (or 'overall') *C*-index.[29 30] It shows how frequently an earlier-event participant has a poorer prediction than a later-or-never-event participant. In other words, it is the concordance between risk score and event time. It is calculated across all pairs of participants where the time order of the pair is deducible from the 9-year data window.

It is noted that in the ROC analysis it was not possible to add two time-dependent covariates, which were needed to compensate for the fact that both age and log(OPG/ng/L) violated the assumption of proportional hazard. However, the output obtainable from the SAS procedure did not allow the inclusion of time-dependent covariates.

2 Patient and public involvement

There was no direct patient involvement in the design of the trial, but the majority of the investigators had daily contact with patients comparable to those included in the trial and therefore knew their needs and preferences well. Moreover, there were patient representatives as part of the regional ethics committee approving the trial.

1 RESULTS

Table 1 presents an overview of the covariates expected to be available from patients with stable CV disease during clinical routine work ('standard predictors') plus the 12 newer biochemical quantities under investigation. The data revealed that at 3 years, 2073 (94.2%) were still alive and 1826 (83.0%) had not yet suffered a composite outcome. At 6 years, 1758 (79.9%) were still alive and 1261 (57.3%) had not yet suffered a composite outcome. At 9 years, the numbers were 1487 (67.6%) and 969 (44.0%).

Out of 2099 placebo patients, 1998 had complete biochemical data. As Little's test[31] had p=0.49, suggesting that the values were missing completely at random, we used complete case analyses in the following.

Two of the 12 newer biomarkers (log(Calprotectin) and log(Cathepsin-S)) did not contribute significantly (p>0.01) to the prediction of any of the two outcomes, neither when used in combination with the 'standard predictors' nor when used alone (see online supplementary tables 1S and 2S). They were therefore removed from the subsequent analyses. In the analysis of log(OPG/ng/L), we found that the assumption of proportional hazard was significantly violated. This was remedied when we included the time-dependent covariate log(OPG/ng/L)*time/year in the subsequent regression equation (see online supplementary table 2S). The latter equation now included the 'standard predictors', plus the remaining 10 newer biomarkers and the above-mentioned time-dependent covariates. It appears from online supplementary table 2S that only log(pro-BNP/ng/L), log(hs-cTnT/ng/L) and log(OPG/ng/L)*time/year contributed significantly to the prediction.

Table 2 (see also online supplementary tables 3S and 4S) compares the number and percentages of correct predictions between various prediction models. In each model, predictions were made at 3, 6 and 9 years for each of the two outcomes (death and the composite). Model 1 shows the results obtained using a model void of covariates; 79.8% of the predictions were correct for the outcome death and 63.2% for the composite outcome. Model 2 shows the results obtained when model 1 was augmented by the 'standard predictors'. Now the per cent correct predictions have been improved by 83.3%–79.8%=3.5% for the outcome death and 68.4%–63.2%=5.2% for the composite outcome. When model 2 was improved by adding the 10 newer biomarkers, the additional gain in correct predictions amounted to 1.4% for death and 1.3% for the composite outcome.

Table 1 Distributions of demographics, previous history, current medication, standard biochemical predictors and newer biochemical predictors in 2199 placebo receiving patients from the CLARICOR trial

Quantity	Distribution
Demographics and previous history	
Sex (male) N (%)	1518 (69.0%)
Age/year mean (SD)	65.2 (10.4)
Smoking status N (%)	
Smokers	753 (34.2%)
Ex-smokers	1011 (46.0%)
Never smoked	435 (19.8%)
Hypertension N (%)	883 (40.2%)
Diabetes N (%)	337 (15.3%)
Previous AMI N (%)	1494 (67.9%)
Current medication	
Aspirin N (%)	1937 (88.1%)
Beta-blocker N (%)	681 (31.0%)
Calcium-antagonist N (%)	772 (35.1%)
ACE-inhibitor N (%)	577 (26.3%)
Long-lasting nitrate N (%)	457 (20.8%)
Diuretics N (%)	773 (35.2%)
Digoxin N (%)	126 (5.7%)
Statins N (%)	904 (41.1%)
Anti-arrhythmic drugs N (%)	51 (2.3%)
Standard biochemical predictors	
log(CRP/mg/L) mean (SD) N*	1.03 (1.12) 2159
ApoA1/mg/dL mean (SD) N	1.70 (0.34) 2076
log(ApoB/mg/dL) mean (SD) N	0.16 (0.27) 2075
Chol-HDL/mmol/L mean (SD) N	1.02 (0.32) 2074
Chol-LDL/mmol/L mean (SD) N	2.56 (0.72) 2079
log(Cholesterol/mmol/L) mean (SD) N	1.73 (0.20) 2075
log(Triglyceride/mmol/L) mean (SD) N	0.73 (0.53) 2078
Glomerular filtration rate (GFR/mL/min) mean (SD) N	71.8 (19.2) 2079
Newer biochemical predictors	
log(pro-BNP/ng/L) mean (SD) N	5.26 (1.37) 2149
log(hs-cTnT/ng/L) mean (SD) N	2.01 (0.78) 2111
log(Endostatin/ng/mL) mean (SD) N	10.3 (0.34) 2121
log(OPG)/ng/L) mean (SD) N	7.49 (0.40) 2108
log(TNFR1/pg/mL) mean (SD) N	7.40 (0.40) 2120
log(TNFR2/pg/mL) mean (SD) N	8.54 (0.33) 2120
PAPP-A ≥4 mIU/L count (%) N	288 (13.1%) 2140
log(YKL40/µg/L) mean (SD) N	4.75 (0.66) 2163
log(NGAL/ng/L) mean (SD) N	11.6 (0.46) 2121
log(Cathepsin-B/µg/L) mean (SD) N	10.6 (0.45) 2120
log(Cathepsin-S/µg/L) mean (SD) N	9.48 (0.27) 2121
log(Calprotectin/mg/L) mean (SD) N	0.77 (0.59) 2086

*The value of N varies because the laboratory tests have missing values (mostly due to storage problems). log: natural logarithm.
AMI, acute myocardial infarction; CRP, C reactive protein; HDL, high-density lipoprotein; hs-cTnT, high-sensitive assay cardiac troponin T; LDL, low-density lipoprotein; NGAL, neutrophil gelatinase-associated lipocalin; OPG, osteoprotegerin; PAPP-A, pregnancy-associated plasma protein A; pro-BNP, serum N-terminal pro-B-type natriuretic peptide; TNFR1, tumour necrosis factor receptor 1; TNFR2, tumour necrosis factor receptor 1.

Using the parametric model in place of the Cox model, we obtained quite similar results (see online supplementary tables 3S and 4S and online supplementary figure 1S A–B). The same was true if we only included log(-pro-BNP/ng/L), log(hs-cTnT/ng/L) and log(OPG/ng/L) instead of all 10 biomarkers when the Cox model was used (see online supplementary tables 3S and 4S).

Table 3 summarises the ROC analyses. For prediction of the composite outcome (yes/no), the area under the ROC increases from 0.711 to 0.732 when the 10 novel biomarkers are added to the 'standard predictors', but almost all the marker information is contained in log(hs-cTnT/ng/L) and log(pro-BNP/ng/L) (AUC=0.730). The 'dynamic' *C*-index values are smaller as prediction of event times is more difficult, but the gains are similar. All-cause death shows the same general pattern.

1 DISCUSSION

In this study, we assessed the combined value of 12 newer biomarkers not routinely used in clinical work to predict all-cause death and a composite outcome (AMI, UAP, CeVD or all-cause death). We used a cut value of predicted risk=0.5 to separate correct predictions of the observed patient status from incorrect ones. When we combined the biomarkers with the 'standard predictors' routinely available for a general practitioner when he/she meets a patient with stable coronary artery disease (CAD), 84.7% of the survival status were correctly predicted. In case of the composite outcome the number was 68.4%. In both cases, the combined contribution of the newer biomarkers amounted to <1.5%.

Our patients resemble those of The Prospective Observational Longitudinal Registry of Patients with Stable Coronary Heart Disease (CLARIFY) study,[32] which enrolled 20 291 patients. The CLARIFY patients had been observed with a median of 24.1 months. However, enrolment took place 10 years later than in the CLARICOR trial and the incidence of CV deaths or myocardial infarctions in these patients was considerably lower,[32] probably reflecting improved quality of treatment and more frequent statin treatment in the CLARIFY patients (84% compared with only 41% in the CLARICOR material). So, the age of our material is a weakness.

In our present study, we are using our data to develop a prediction model. Then we evaluate the performance using the same data that we used to develop the model. Clearly, this is bound to produce overly optimistic results compared with testing our model using independent data. But we argue that the aim of this study was not to present a prediction model but to assess the newer biomarkers' contribution to model performance when added on top of routinely available clinical and laboratory data. Therefore, if tested on independent data, the contribution of the newer biomarkers to prognosis of patients with stable CAD are likely going to be worse than observed here.

Table 2 The two outcomes (1) all-cause death and (2) the composite outcome of AMI, UAP, CeVD or all-cause death were studied

Model and covariates included in model	Total number of predictions made per outcome	Number and per cent of correct predictions of events	
		All-cause death N (%)	Composite of AMI, UAP, CeVD or all-cause death N (%)
Model 1: Cox model void of covariates	5972	4768 (79.8)	3773 (63.2)
Model 2: Cox model with 'Standard predictors (SP)' added to model	5972	4977 (83.3)	4084 (68.4)
Model 3: Cox model with SP+10 newer biomarkers added to model	5972	5056 (84.7)	4165 (69.9)

AMI, acute myocardial infarction; CeVD, cerebrovascular disease; UAP, unstable angina pectoris.

2 Methodology

Regarding our methodology, the performance statistics reported here are minimal, but they suffice to show that the results are meagre. Prediction at 3, 6 and 9 years covers the follow-up as well as a sophisticated integral over continuous time.

2 Strengths

The strengths of the CLARICOR trial are the size of the patient population, the long duration of follow-up, few losses to follow-up (1%), the ethnic homogeneity of the patient population (most being Caucasians), rarity of missing values, with focus on an operationally defined, homogeneous and relevant patient category. The design implies that the patients are sampled at random, presumably uneventful, time points during their stable state (as defined by the CLARICOR trial).

Table 3 *C*-indices

	Binary outcome C (AUC)	Dynamic C
	Observed (predicted)*	Observed†
Composite outcome‡ (1115 events)		
SP only	0.711 (0.707)	0.640
The 10 newer markers and SP	0.732 (0.732)	0.657
log(hs-cTnT/ng/L)+log(pro-BNP/ng/L)+SP	0.730 (0.730)	0.656
All-cause death (644 deaths)		
SP only	0.792 (0.793)	0.737
The 10 newer markers and SP	0.824 (0.816)	0.765
log(hs-cTnT/ng/L)+log(pro-BNP/ng/L)+SP	0.821 (0.813)	0.762

Cox model estimates applied to the 0–9 years follow-up window (n=1998).
*The 'observed' AUCs summarise a ROC plot of cumulative events against cumulative non-events, with cumulation from large to small estimated risks. The corresponding 'predicted' AUC cumulates the predicted risks instead. Discrepancies between the two curves would suggest a model failure (calibration problems). The curves (not shown) were practically identical.
†Analogous concordance rate between time to event and predicted risk.
‡Composite outcome: first occurrence of acute myocardial infarction, unstable angina pectoris, cerebrovascular disease or death (table 1.
AUC, area under the curve; hs-cTnT, high-sensitive assay cardiac troponin T; ROC, receiver operating characteristics; SP, standard predictors.

2 Limitations

Among those 7586 patients who declined our invitation to visit a cardiology centre, many must have been eligible for the CLARICOR trial, and we do not know how they looked and fared. With a response rate about 50%, the cohort could represent a prognostic elite if responders were mostly mobile and health-conscious patients. So, selection bias cannot be excluded.

Furthermore, users of these data should remain aware of one feature: patients if any who became eligible for the CLARICOR trial during the period 1993 to 1999 and then died before August 1999 are absent. Thus, our data do not represent patients as they enter a stable disease state (as delimited by CLARICOR exclusion criteria); instead, they may be regarded as community patients (subject to some self-selection) seen by their physician or at an outpatient clinic on a random date during their stable state.

The patients recruited for the CLARICOR trial were diagnosed with CAD about 20 years ago. Because of the developments in treatment and rehabilitation, there has been a very significant and gradual improvement in the prognosis of such patients as shown in national data.[33] Given these uncertainties, prognostic findings in the CLARICOR cohort may not be directly applied to present-day patients. However, the overall, somewhat disappointing, picture presented by the predictive performance of standard[1] and newer biochemical predictors studied 10–20 years ago would hardly be much different if studied today.

Potential weaknesses of the present cohort within the context of prognostication of patients with stable CAD as here defined include the fact that only questionnaire data were collected at randomisation. No data are available concerning left ventricle function, body mass index, blood pressure and general health. These shortcomings are mitigated by the fact that, by design, the present study

sees the patient in a situation where (s)he visits a physician for reasons unrelated to the coronary disease, as already stressed. In such situations, counselling and decisions must typically be made without access to echocardiography or other special investigations. Furthermore, if this information had been available, the prognostic gain we study would probably have been still poorer. Moreover, we included age, sex, hypertension, prior myocardial infarction, information about current medication which has previously been shown to be a fair replacement for prognostication instead of left ventricular ejection fraction.[34]

It is noted that the patients studied by us were all in a stable state of their disease, without cardiac complaints. Therefore, one should not conclude from this study that the biomarkers studied here may not be useful in many other clinical contexts, although biomarkers have been shown to of modest help in evaluating CV risk assessment in asymptomatic people not suffering from CAD.[35]

1 CONCLUSIONS

In the present clinical context, the contribution of the 12 biomarkers not yet used in clinical routine work proved to be minimal. Furthermore, of the 10 statistically promising novel biomarkers all could be replaced by hs-cTnT and pro-BNP, possibly supplemented by OPG.

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Contributors PW, JH, JCJ and CG contributed substantially to the concept and design and drafted the manuscript, PW and JH contributed equally to this paper, and conducted the statistical analyses. AL and JÄ conducted the analysis of lipids and creatinine. PW, JCJ, JH, GBJ, EK, AS, JK, HJK, KKI, MB, AL, JÄ and CG revised the manuscript critically for important intellectual content, gave final approval of version to be published and agreed to be accountable for all aspects of the work in assuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. PW, JCJ, JH, GBJ, EK, AS, JK, HJK, KKI, MB, AL, JÄ and CG contributed substantially to the interpretation of the data.

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Patient consent for publication Not required.

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Data availability statement Data will be available in a public, open access repository. All pertinent anonymised data will be uploaded at ZENODO (http://zenodo.org/) when the individual manuscripts have been published.

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