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Combined prognostic value of 12 novel cardiological biomarkers. A 10-year follow-up of placebo receiving patients with stable coronary disease sampled at random times during their disease course

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Combined prognostic value of 12 novel cardiological biomarkers. A 10-year follow-up of placebo receiving patients with stable coronary disease sampled at random times during their disease course

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ABSTRACT

Objective

We investigated whether 12 novel circulating biomarkers were capable, when added to 'standard predictors' available in general practice, of improving the 10-year prediction of cardiovascular events and mortality in 2199 patients with stable coronary heart disease (CLARICOR trial placebo group). The patients participated as placebo receiving patients in the randomised CLARICOR trial at a random time in their disease trajectory.

Methods

The predictors were based on demographic information from hospital files, interviews, and blood samples collected at entry into the trial. We studied the prognosis for all-cause death and for the composite outcome of either all-cause death, myocardial infarction, unstable angina, or cerebro-vascular disease. We estimated each participant's survival probability at specified time points and report the correct prediction rate, 0.5 being taken as cut point for the estimated survival probability, with Cox regression analysis.

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 ten biomarkers (high-sensitive assay cardiac troponin T; neutrophil gelatinase-associated lipocalin; osteoprotegerin; N-terminal pro-B-type natriuretic peptide;

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tumor necrosis factor receptor 1 and 2; PAPP-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 heart disease, the addition of 10 novel biomarkers to the prediction model improved the correct prediction of all-cause death and the composite outcome by less than 1.5%.

Trial registration: ClinicalTrials.gov, NCT00121550. Date of registration 13 July 2005

Date of enrolment of first participant 12 October 1999

Keywords: CLARICOR, cardiovascular disease, cardiovascular risk prediction, ischaemic heart disease, predictors, mortality.

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INTRODUCTION

In a previous paper[1] and in accordance with our published and peer-reviewed statistical analysis plan,[2] we assessed the prognostic value of quantities, readily available during clinical routine work, when a patient with stable coronary heart disease is seen by the practicing physician or at an outpatient clinic without renewed cardiac complaints.

We examined predictors for all-cause death alone and a composite outcome (all-cause death, acute myocardial infarction (AMI), unstable angina pectoris (UAP), and cerebro-vascular disease (CeVD)). We used an operational definition of the term 'stable coronary disease' adapted from the 'clarithromycin for patients with stable coronary heart disease' (CLARICOR) trial[3,4] which covers a common and important class of patients, used the data from the placebo patients to develop the prognostic model, and tested how well it predicted the actual outcomes during the 10-year period following randomisation.

The frequency of correct status predictions increased by 3.5% for all-cause death and by 5.2% for the composite outcome when the routinely available predictors shown in table 1 were used.One might speculate that this rather modest result could be improved by combining the routinely available predictors with some of those biomarkers which from a pathophysiological point of view seem promising.

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Within the last years many such biomarkers e.g.[5,6] have appeared, all claimed to add some prognostic information in patients with stable coronary artery heart disease. In most cases the information has been evaluated in addition to that of routinely available clinical and laboratory information. We and others have tested the individual importance of many of these biomarkers and in many studies statistical inference supports the view that biomarkers may improve the prediction[7-12].

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 etc.

MATERIAL

The patients

The study population is the placebo patients from the CLARICOR-study.[3,4] Patients aged 18 to 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-days trial of clarithromycin versus placebo.[3,4] Out of the 4372 who were randomised during October 1999 through April 2000, 2199 were in the placebo group.

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

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To secure that only patients were in a stable state of their coronary heart disease, patients were excluded if they fulfilled one or more of the following conditions: (1) had suffered from acute myocardial infarction or unstable angina pectoris within the previous 3 months; (2) had had intra-coronary interventions within the previous 6 months; (3) had impaired renal function; (4) had hepatic dysfunction; (5) had congestive heart failure (New York Heart Association (NYHA) 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.

All patients were followed up until death or end of the CLARICOR trial on DEC 31, 2009 using public Danish registries. Only two of the 2199 participants were lost track of, due to emigration.

The predictors

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

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 – 80 degrees C. The quantities measured include lipoproteins,[16] high-sensitivity-C-reactive-protein/mg/L (hs-CRP/mg/L),[7] and glomerular filtration /rate/mL/min (GFR/mL/min) using creatinine.[17] Along with variables already mentioned,

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these quantities are those collectively referred to as 'standard predictors' and specified in Table 1.

Biomarkers included as newer biomarkers were YKL40/µg/L;[8] high-sensitive assay cardiac troponin T/ng/L (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] N-terminal pro-B-type natriuretic peptide/ng/L (proBNP/ng/L);[9] cathepsin-B/µg/L;[6,18] ;endostatin/ng/mL;[19] cathepsin-S/µg/L;[6,20] soluble TNF receptor 1/pg/mL; and soluble TNF receptor 2/pg/mL; (sTNFR1/pg./mL and sTNFR2/pg/mL);[5,21] neutrophil gelatinase-associated lipocalin/ng/L (NGAL/ng/L);[22] calprotectin/mg/L;[11] and osteoprotegerin/ng/L; (OPG/ng/L).[12] Due to storage problems some marker data are missing on some patients, as listed in Table 1.

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 December 31, 2009. The public registers have an almost 100% coverage and the quality of these are 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]

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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: acute myocardial infarction (AMI), unstable angina pectoris (UAP), cerebrovascular disease (CeVD), or all-cause death.

METHODS

Statistical analysis

The statistical principles and techniques used have previously been published.[1,2] We used Cox regressions (SAS 9.4) where all analyses that included covariates were stratified by centre. We also analysed data using a parametric, accelerated failure-time model using the generalized gamma model of error (see Figure 1).[27] A significance level of 0.01 was used to pinpoint empirical trends worthy of note. The logarithms of the present text are natural logarithms, and all Cox hazard ratios refer a unit increase, i.e. a factor e = 2.72 concentration increase whenever the predictor is a log (serum level/unit).

Biomarkers with a hazard rate with P value $\geq$ 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 percent 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]

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RESULTS

Table 1 presents an overview of the covariates expected to be available from stable cardiovascular disease patients during clinical routine work ('standard predictors') plus the 12 newer biochemical quantities under investigation. The mean observation time was 8.323 year. The total number of patient observation years was 16630 year. 738 (63.1%) patients died during the observation period. 1204 (60.3%) experienced a composite outcome.

Out of 2199 placebo patients, 1998 had complete biochemical data. As Little's test[28] had P = 0.49, suggesting that the values were missing completely at random, we used complete case analyses in the following. The data revealed that at 3 years, 2073 (94.3%) 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 (44.9%).

Inferential impact of the newer biomarkers

As the proportional hazard's assumption was violated for age[29] and age interacted significantly with time since randomisation, we included an interaction between age at entry and time (since randomisation) in the inference analyses.

Table 2 shows the results of a Cox regression of all-cause death on each of the 12 biomarkers when the biomarker was used alone as a covariate (columns 2 through 4), and when it was used in combination with the 'standard predictors' (columns 5 through 7).

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Columns 8 through 10 in Table 2 shows the result of a regression of the outcome on the 'standard predictors' and the 10 best biochemical predictors. Now only log (proBNP /ng/L) and log(hs-cTnT/ng/L) have a HR significantly ($P < 0.01$) different from 1. Log(calprotectin/mg/L) and log(cathepsin-S/µg/L) did not have an inferential impact ($P < 0.01$ not attained), not even when used alone.

Table 3 corresponds to Table 2 except that the outcome is the composite outcome. It is noted that a time-dependent covariate is now included because log (OPG/ng/L) violated the proportional hazard assumption. This was remedied by including the covariate log (OPG/ng/L) ·time/year. It is seen that when all the biomarkers were included in the Cox analysis log(OPG/ng/L)·time/year, log(proBNP/ng/L), and log(hs-cTnT/ng/L) were the only ones which had a P value below the threshold of 0.01. Again log(calprotectin/mg/L) and log(cathepsin-S/µg/L) could be excluded from the final analysis, the result of which is shown in columns 8 through 10.

Practical impact of the novel biomarkers

The results of the predictions of survival status made at 3 years, at 6 years, and at 9 years following randomisation in the 2199 placebo patients are summarised in Table 4.

When the 'standard predictors' were included as covariates (column 5) for all-cause mortality, 83.3% of the predictions were correct. Adding the 10 newer biochemical predictors (column 6) the percentage was increased by 1.4% to 84.7%. It is noted that the results obtained with the parametric model (column 7) are not dramatically different when this theoretically equally valid model is used. When only the two significant predictors (log

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(proBNP/ng/L) and log (hs-cTnT/ng/L)) were used in the Cox model in place of all 10 (column 8), the results were practically unaffected (compare columns 8 and 6).

Table 5 shows the results corresponding to Table 4 obtained when the composite outcome was used. Including the 'standard predictors' in the model increases the percent correct predictions from 63.2 (see column 4, Table 5) to 68.4 (see column 5, Table 5), i.e. an increase of 5.2%. Adding the 10 newer biomarkers to the model increases the number of correct predictions by 1.3%. Using the parametric model does not change the results appreciably and neither does a reduction of the biomarkers to include only the three significant ones.

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 P=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 heart disease, 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 less than 2%.

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Most of the studies we have identified in the literature only include small study samples, [e.g.20] were developed in patients with acute coronary syndromes, [e.g.21] or had a short follow-up.[e.g. 22]

Our patients resemble those of The Prospective Observational Longitudinal Registry of Patients with Stable Coronary Heart Disease (CLARIFY) study[30] which enrolled 20.291 patients. The CLARIFY patients had been observed with a median of 24.1 months. However, enrollment took place 10 years later than in the CLARICOR trial and the incidence of cardiovascular deaths or myocardial infarctions in these patients was considerably lower,[30] probably reflecting improved quality of treatment and more frequent statin treatment in the CLARIFY patients (84% compared to only 41% in the CLARICOR material).

In our present study, we are using our data to develop a prediction model. Then we use the same data that we used to develop the model. Clearly this is bound to produce overly optimistic results compared to 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 coronary heart disease are likely going to be worse than observed here.

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 would a sophisticated integral over continuous time.

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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 (0.1%), 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).

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.

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The patients recruited for the CLARICOR trial were diagnosed with coronary heart disease 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.[31] 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 stable coronary heart disease patients 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.[32]

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

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been shown to of modest help in evaluating cardiovascular risk assessment in asymptomatic people not suffering from CAD.[33]

Conclusions In the present clinical context the contribution of the 12 biomarkers not yet used in clinical routine work proved to be minimal. Furthermore, 9 of the 10 novel biomarkers could all except for osteoprotegerin be replaced by hs-cTnT and proBNP.

Strengths and limitations of this study

- 9 years duration of follow-up
- Patients sampled at random times during their stable state
- Only 0.1% losses to follow-up
- Patients recruited about 20 years ago
- Only questionnaire information data were collected at randomisation

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. The public involvement was trough the approvals given by the regional ethics committee (KF 01-076/99 and journal no. H-12012125), the Danish Medicines Agency (2612-975), and the Danish Data Protection Agency (1999-1200-174).

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What is already known about this subject?

Patients with stable coronary artery disease are at an increased risk of death or non-fatal cardiovascular incidents.

What does this study add?

New knowledge regarding the long-term impact of 12 newer biochemical factors not used in clinical routine for the prediction of all-cause death or of non-fatal cardiovascular incidents or all-cause mortality in patients with stable coronary heart disease ascertained at baseline review not prompted by renewed cardiac complaints.

Ten of the 12 biochemical factors did show a highly significant expected association with long-term course, but the practical prognostic impact is weak with a combined improvement of the number of correctly predicted patient status of less than 1.5%. For useable prognostic differentiation, stronger clinical markers are needed.

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Abbreviations

AMI: acute myocardial infarct; Apo A1: apoprotein A1; Apo B: apoprotein B; CeVD: cerebro-vascular disease; Chol-HDL: cholesterol high density lipoprotein; Chol-LDL: cholesterol low density lipoprotein; CLARICOR: Clarithromycin for patients with stable coronary heart disease; CRP: c-reactive protein; GFR: Glomerular filtration rate; hs-cTnT: High-sensitive assay cardiac troponin T; NGAL: neutrophil gelatinase-associated lipocalin; NYHA: New York Heart Association; OPG: osteoprotegerin; PREMAC: Predictors for major cardiovascular outcomes in stable ischaemic heart disease; proBNP: N-terminal pro-B-type natriuretic peptide;

TNFR1: tumor necrosis factor receptor 1; TNFR2: tumor necrosis factor receptor 2; UAP: unstable angina pectoris.

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Availability of data and materials

All pertinent anonymised data will be uploaded at ZENODO (http://zenodo.org/) when the individual manuscripts have been published.

Authors' contributions

PW, JH, JCJ, and CG contributed substantially to the concept and design and drafted the manuscript. PW and JH conducted the statistical analyses. AL and JÄ conducted the analysis of lipids and creatinine. All authors 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.

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Competing interests

The authors declare that they have no competing interests.

Ethics approval and consent to participate was given by VEKKF01-076/99; Danish Medicines Agency 2612-975; Danish Data Protection Agency 1999-1200-174; VEK H-B-2009-015.

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REFERENCES

1. Winkel P, Jakobsen JC, Hilden J, et al. Prognostic value of routinely available data in patients with stable coronary heart disease. A 10-year follow-up of patients sampled at random times during their disease course. Open Heart 2018:e000808. Doi:10.1136/openhrt-2018-000808

2. Winkel P, Jakobsen JC, Hilden J, Lange T, Jensen GB, Kjøller E, et al: Predictors for major cardiovascular outcomes in stable ischaemic heart disease (PREMAC): statistical analysis plan for data originating from the CLARICOR (clarithromycin for patients with stable coronary heart disease) trial: Diagnostic and Prognostic Research (2017) 1:10 DOI 10.1186/s41512-017-0009-y.

3. Jespersen CM, Als-Nielsen B, Damgaard M, Hansen JF, Hansen S, Helø OH, et al: Randomised placebo controlled multicentre trial to assess short term clarithromycin for patients with stable coronary heart disease: CLARICOR trial. BMJ. 2006; 332:22–7.

4. Hansen S, Als-Nielsen B, Damgaard M, Helø OH, Petersen L, Jespersen CM, et al: Intervention with clarithromycin in patients with stable coronary heart disease: the CLARICOR trial design. Heart Drug. 2001; 1:14–9.

5. Carlsson AC, Ruge T, Kjøller E, Hilden J, Kolmos HJ, Sajadieh A o. a. 10-year associations between tumor necrosis factor receptors 1 and 2 and cardiovascular events in patients with stable coronary heart disease: a CLARICOR (effect of clarithromycin on mortality and morbidity in patients with coronary heart disease) trial substudy. Journal of the American Heart Association 2018 apr 23;7(9): e 008299 Doi:10. 116/JAHA 127.008299

6. Wuopio J, Hilden J, Bring C, Kastrup J, Sajadieh A, Jensen GB o.a. Cathepsin B and S as markers for cardiovascular risk and all-cause mortality in patients with stable coronary heart disease during 10 years: a CLARICOR trial substudy. Atherosclerosis. 2018 nov 1:278: 97 to 102. Tilgængelig fra, DOI: 10. 1016/j atherosclerosis. 2018.09.006

7. Harutyunyan MJ, Mathiasen AB, Winkel P, Gøtze JP, Hansen JF, Hildebrandt P, et al: High-sensitivity C-reactive protein and N-terminal pro-B-type natriuretic peptide in

For peer review only

patients with stable coronary heart disease: a prognostic study within the CLARICOR trial. Scand J Clin Lab Invest. 2011; 71:52–62.

8. Kastrup J, Johansen JS, Winkel P, Hansen JF, Hildebrandt P, Jensen GB, et al.High serum YKL40 concentration is associated with cardiovascular and all-cause mortality in patients with stable coronary artery disease. Eur. Heart J. 2009; 30:1066-72.

9. Lyngbaek S, Winkel P, Gotze JP, Kastrup J, Gluud C, Kolmos HJ, et al. Risk stratification in stable coronary artery disease is possible at cardiac troponin levels below conventional detection and is improved by use of N-terminal pro-B-type natriuretic peptide. European Journal of Preventive Cardiology 2014; 21:1275-1284.

10. Iversen KK, Teisner B, Winkel P, Gluud C, Kjoller E, Kolmos HJ, et al. Pregnancy associated plasma protein-A as a marker for myocardial infarction and death in patients with stable coronary artery disease: a prognostic study within the CLARICOR Trial. Atherosclerosis 2011; 214:203-208.

11. Larsen SB, Grove EL, Pareek M, Kristensen SD, Hvas AM. Calprotectin and platelet aggregation in patients with stable coronary artery disease. PloS ONE 2015; 10(5):e0125992.

12. Bjerre M, Hilden J, Kastrup J, Skoog M, Hansen JF, Kolmos HJ, et al. Osteoprotegerin independently predicts mortality in patients with stable coronary artery disease: the CLARICOR trial. Scandinavian Journal of Clinical and Laboratory Investigation 2014; 74:657-664.

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13. Winkel P, Hilden J, Hansen JF, Kastrup J, Kolmos HJ, Kjoller E, et al. Clarithromycin for stable coronary heart disease increases all-cause and cardiovascular mortality and cerebrovascular morbidity over 10years in the CLARICOR randomised, blinded clinical trial. International Journal of Cardiology 2015, 182:459-465.

14. Winkel P, Hilden J, Fischer Hansen J, Hildebrandt P, Kastrup J, Kolmos HJ, et al: Excess sudden cardiac deaths after short-term clarithromycin administration in the CLARICOR trial: why is this so, and why are statins protective? Cardiology. 2011; 118:63–67.

15. Gluud C, Als-Nielsen B, Damgaard M, Fischer Hansen J, Hansen S, Helø OH, et al: Clarithromycin for 2 weeks for stable coronary heart disease: 6-year follow-up of the CLARICOR randomized trial and updated meta-analysis of antibiotics for coronary heart disease. Cardiology. 2008; 111:280

16. Helmersson-Karlqvist J, Ridefelt P, Lind L, Larsson A. Reference values for 34 frequently used laboratory tests in 80-year-old men and women. Maturitas. 2016; 92: 97-101.

17. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al: Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012;367:20–9.

18. Wang N, Bai X, Jin B, Han W, Sun X, Chen X. The association of serum cathepsin-B concentration with age-related cardiovascular-renal subclinical state in a healthy Chinese population. Arch Gerontol Geriatr. 2016; 65:146-55.

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19. Arnlov J, Ruge T, Ingelsson E, Sundstrom J, Lind L. Serum endostatin and risk of mortality in the elderly: findings from 2 community-based cohorts. Arterioscler Thromb Vasc Biol.2013, 33: 2689-95.

20. Jobs E, Ingelsson E, Risérus U, Nerpin E, Jobs M, Sundström J, et al. Association between serum cathepsin S and mortality in older adults. JAMA. 2011; 306:1113-21.

21. Carlsson AC, Larsson TE, Helmersson-Karlqvist J, Larsson A, Lind L, Ärnlöv J. Soluble TNF receptors and kidney dysfunction in the elderly. J Am Soc Nephrol. 2014;25:1313-20.

22. Helmersson-Karlqvist J, Larsson A, Carlsson AC, Venge P, Sundstrom J, Ingelsson E, et al. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is associated with mortality in a community-based cohort of older Swedish men. Atherosclerosis 2013; 227:408-13.

23. Kjøller E, Hilden J, Winkel P, Frandsen NJ, Galatius S, Jensen G, et al: Good interobserver agreement was attainable on outcome adjudication in patients with stable coronary heart disease. J Clin Epidemiol. 2012; 65:444–53.

24. Kjøller E, Hilden J, Winkel P, Galatius S, Frandsen NJ, Jensen GB, et al: Agreement between public register and adjudication committee outcome in a cardiovascular randomized clinical trial. Am Heart J. 2014; 168:197–204.ok

25. Helweg-Larsen K: The Danish register of causes of death. Scand J Public Health. 2011;39(7 30. Suppl):26-9.

For peer review only

26. Lynge E, Sandegaard JL, Rebolj M: The Danish National Patient Register. Scand J Public Health. 2011;39(7 suppl):30–3.

27. Allison PD: Survival Analysis Using SAS: A Practical Guide, second edition 2010 SAS institute Inc. Cary, NC, USA.

28. Little R: A test of missing completely at random for multivariate data with missing values. J Am Stat Assoc. 1988; 83:1198–1202.

29. Lin DY, Wei LJ, Ying Z: Checking the Cox model with cumulative sums of martingale-based residuals. Biometrika. 1993; 80:557–72.

30. Steg PG, Greenlaw N, Tendera M, et al. Prevalence of anginal symptoms and myocardial ischemia and their effect on clinical outcomes in outpatients with stable coronary artery disease. Data from the International Observational CLARIFY Registry. JAMA Intern Med. 2014;174(10):1651-1659. doi:10.1001/jamainternmed.2014.3773

31. Schmidt M, Jacobsen JB, Lash TL, Bøtker HE, Sørensen HT: 25-year trends in first time hospitalisation for acute myocardial infarction, subsequent short and long-term mortality, and the prognostic impact of sex and comorbidity: a Danish nationwide cohort study. BMJ. 2012;344: e356.

32. Solomon SD, Claggett B,Desai AS, *et al*. Influence of ejection fraction on outcomes and efficacy of sacubitril/valsartan (lcz696) in heart failure with reduced ejection fraction. Circulation 2016;9:e00274.

33. Scott IA. Evaluating cardiovascular risk assessment in asymptomatic people.BMJ 2009:a2844doi:10.1136/bmj.a2844

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Figure 1 A

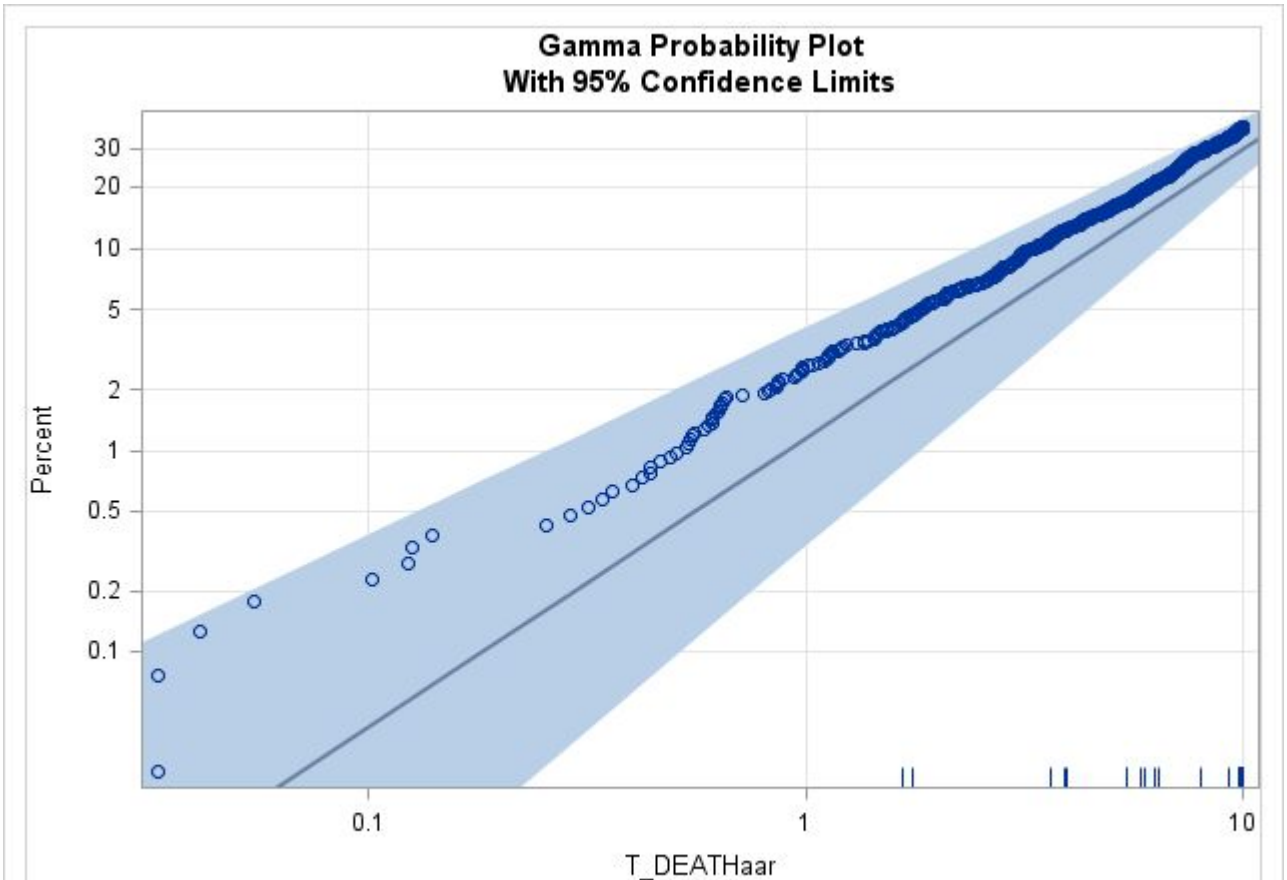
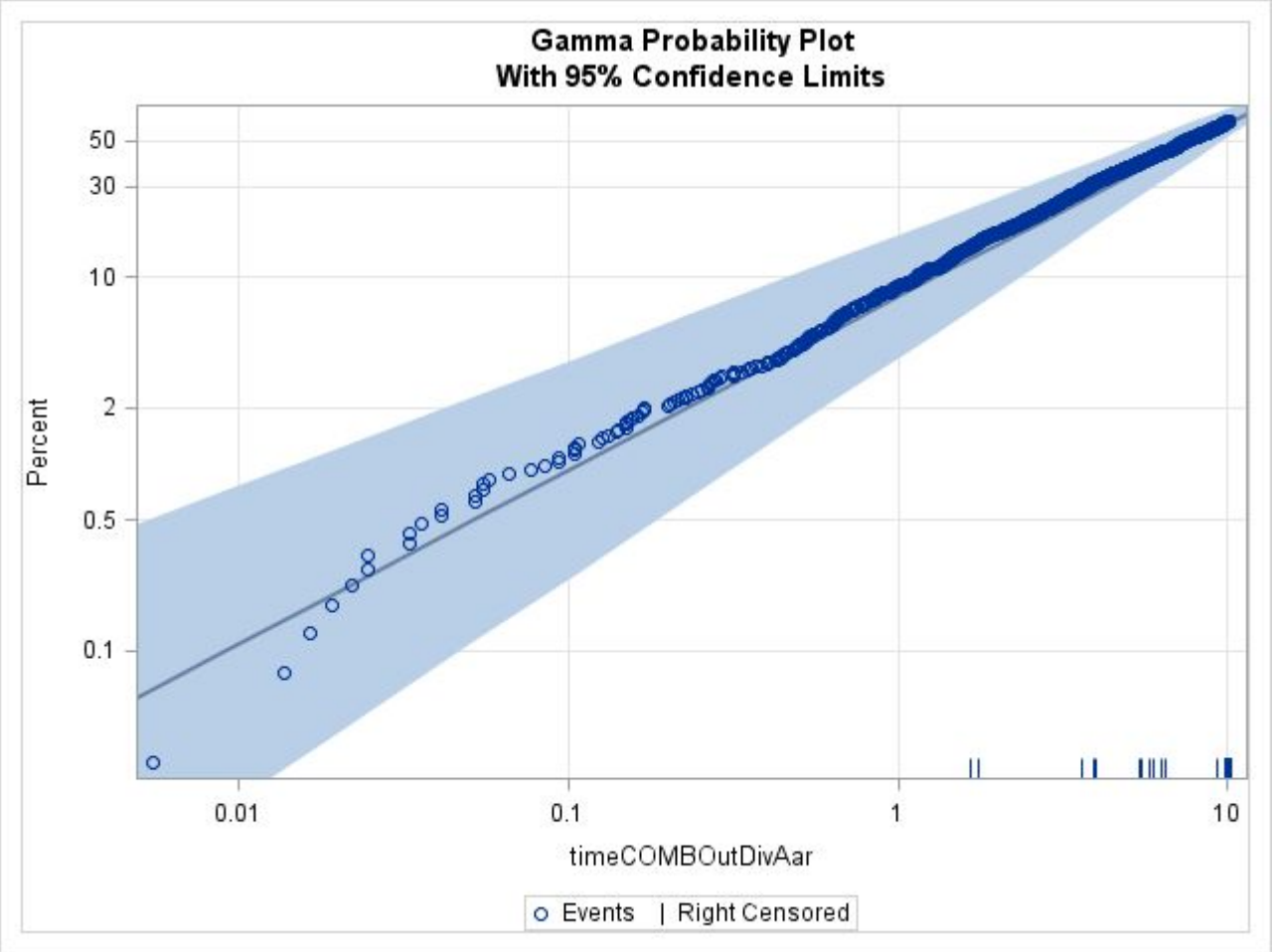

Gamma Probability Plot
With 95% Confidence Limits
Percent
30
20
10
5
2
1
0.5
0.2
0.1
0.1
1
10
T_DEATHaar
Events | Right Censored

Figure 1 B



Legend to figure 1

Figure 1 A Distribution of years to death using the accelerated failure model where the error term is modelled using the general gamma distribution.

Figure 1 B shows similar plot for the distribution of years to composite outcome.

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1

Quantity	Distribution
Demographics and previous history	
Sex (male) N (%)	1518 (69.0%)
Age/year mean (SD)	65.2 (10.4) 2199
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[a]	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 (Tri-glyceride/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 (proBNP/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 ≥ 4mIU/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

Legend to table 1

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.[3]

Abbreviations as in section on abbreviations in the main paper.

FOOTNOTES

a) The value of N varies because the laboratory tests have missing values (mostly due to storage problems). log: natural logarithm.

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Newer biochemical candidate predictor	When candidate predictor is the only predictor included in the model (stratified by centre)			When 'standard predictors' is added to the model (stratified by centre)			When in addition the 10 selected predictors are added to the model (stratified by centre)		
	HR[b]	95% CI	P	HR	95% CI	P	HR	95% CI	P
log (endostatin/ng/mL)	3.49	2.81 to 4.33	<0.0001	1.75	1.34 to 2.27	<0.0001	1.23	0.92 to 1.63	0.16
log (OPG/ng/L)	3.37	2.88 to 3.94	<0.0001	1.68	1.35 to 2.09	<0.0001	1.21	0.97 to 1.63	0.092
log (sTNFR1/pg/mL)	3.80	3.19 to 4.54	<0.0001	1.84	1.46 to 2.33	<0.0001	1.10	0.81 to 1.48	0.55
og (sTNFR2/pg/mL)	5.45	4.40 to 6.76	<0.0001	2.39	1.80 to 3.18	<0.0001	1.43	0.99 to 2.07	0.056
log(proBNP/ng/L)	1.76	1.66 to 1.87	<0.0001	1.44	1.34 to 1.55	<0.0001	1.28	1.19 to 1.39	<0.0001
log(hs-cTnT/ng/L)	2.31	2.16 to 2.47	<0.0001	1.73	1.56 to 1.92	<0.0001	1.46	1.30 to 1.65	<0.0001
PAPP-A_binary[c]	1.84	1.53 to 2.21	<0.0001	1.39	1.15 to 1.68	0.0007	0.85	0.69 to 1.03	0.10
log (YKL40/µg/L)	1.76	1.59 to 1.95	<0.0001	1.32	1.17 to 1.49	<0.0001	1.10	0.97 to 1.25	0.15
log (NGAL/ng/L)	1.33	1.12 to 1.57	0.0011	1.03	0.85 to 1.24	0.78	0.90	0.74 to 1.10	0.30
log(Calprotectin/)	1.08	0.95 to 1.23	0.25	1.02	0.89 to 1.18	0.74	Not included in analysis		
log (Cathepsin-B/µg/L)	2.81	2.40 to 3.28	<0.0001	1.43	1.19 to 1.73	0.0002	1.09	0.89 to 1.33	0.42
log (Cathepsin-S/µg/L)	1.12	0.86 to 1.47	0.40	1.10	0.83 to 1.45	0.51	Not included in analysis		

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Legend to Table 2

All-cause mortality hazard ratios (HR) of newer biochemical predictors not routinely used in clinical work when each of these predictors is used alone (columns 2 to 4), and when it is used in combination with the 'standard predictors' (column 5 to 7). Two of them were then discarded and each of the remaining 10 was assessed when used in combination with the standard predictors and the remaining 9 of the 10 newer biochemical predictors selected among the 12 candidates (columns 8 to 10).

a) The standard predictors are shown in Table 1.
b) Hazard ratio associated with unit increase on log scale, except for PAPP-A (binary).
c) Binary quantity. 1: PAPP-A was ≥ 4 mIU/L, 0: PAPP-A was < 4 mIU/L.

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Newer biochemical candidate predictor	When candidate predictor is the only predictor included in the model (stratified by centre)			When 'standard predictors' is added to the model (stratified by centre)			When in addition the 11[a] selected predictors are added to the model (stratified by centre)		
	HR	95% CI of HR	P	HR	95% CI of HR	P	HR	95% CI of HR	P
log (Endostatin/ng/mL)	2.18	1.84 to 2.58	<0.0001	1.44	1.17 to 1.72	0.0006	1.23	0.99 to 1.54	0.062
log (OPG/ng/L)	1.34	1.05 to 1.71	0.019	0.94	0.70 to 1.26	0.67	0.78	0.58 to 1.04	0.094
log (OPG/ng/L) ·time/year[b]	1.11	1.06 to 1.16	<0.0001	1.09	1.03 to 1.16	0.0022	1.104	1.044 to 1.168	0.0005
log (sTNFR1/pg/mL)	2.14	1.86 to 2.46	<0.0001	1.33	1.11 to 1.60	0.0021	1.05	0.84 to 1.32	0.67
log (sTNFR2/pg/mL)	2.56	2.15 to 3.03	<0.0001	1.49	1.19 to 1.85	0.0004	1.13	0.85 to 1.50	0.40
log (proBNP/ng/L)	1.37	1.31 to 1.44	<0.0001	1.26	1.19 to 1.33	<0.0001	1.18	1.11 to 1.25	<0.0001
log (hs-cTnT/ng/L)	1.83	1.70 to 1.97	<0.0001	1.49	1.35 to 1.64	<0.0001	1.31	1.17 to 1.46	<0.0001
PaPP-A (binary)[c]	1.45	1.24 to 1.70	<0.0001	1.24	1.06 to 1.46	0.0077	0.89	0.75 to 1.05	0.15
log (YKL40/µg/L)	1.35	1.24 to 1.47	<0.0001	1.13	1.03 to 1.24	0.013	1.01	0.91 to 1.11	0.93
log (NGAL/ng/L)	1.23	1.08 to 1.40	0.0023	1.03	0.89 to 1.19	0.73	0.97	0.84 to 1.13	0.74
log (Calprotectin/)	1.06	0.95 to 1.17	0.32	1.00	0.90 to 1.12	0.95	Not included in analysis		
log (cathepsin-B/µg/L)	1.70	1.50 to 1.93	<0.0001	1.17	1.01 to 1.35	0.040	0.99	0.85 to 1.16	0.92
log (cathepsin-S/µg/L)	1.06	0.86 to 1.31	0.59	0.98	0.79 to 1.22	0.88	Not included in analysis		

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Legend to Table 3

The composite outcome (comprising first occurrence of acute myocardial infarction, unstable angina pectoris, cerebro-vascular disease, and death). Hazard ratios of each of 13 biochemical predictors not routinely used in clinical work when each of these predictors is used alone (columns 2 to 4), and when it is used in combination with the 'standard predictors' (column 5 to 7). Two of them were then discarded and each of the remaining 11 was assessed when used in combination with the standard predictors and the remaining 10 of the 11 newer biochemical predictors selected among the 13 candidates (columns 8 to 10).

3

a) Note that now a time dependent covariate has been added [log (OPG/ng/L) · time/year] to the 10 original predictors.

b) log (OPG/ng/L) significantly violated the proportional hazard assumption. We found a significant linear relationship between log (OPG/ng/L) and time since randomisation which may explain the violation. The product of log (OPG/ng/L) and time/year was therefore included in the inference analysis. However, when the Cox model is used for prediction, time dependent covariates are not allowed (SAS 9.4). Therefore, in the latter context we only include log (OPG/ng/L).

c) Binary quantity. 1: PAPP-A was ≥4 mIU/L, 0: PAPP-A was <4 mIU/L.

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(1) Number of predictions made	(2) Time at which prediction was made	(3) Correctly predicted patient status	(4) Data without covariates included Both models N (%)	(5) Data including Standard predictors as covariates Cox model N (%)	(6) Data including Standard predictors + advanced biochemical predictors as covariates Cox model N (%)	(7) Data including Standard predictors + advanced biochemical predictors as covariates Parametric model N (%)	(8) Data including Standard predictors + log (hsTnT) and log(proBNP) as covariates Cox Model N (%)
1996	Three years	Favorable status	1825 (91.4)	1821 (91.2)	1816 (91.0)	1814 (90.9)	1816 (91.0)
		Unfavorable status	0 (0.00)	10 (0.50)	19 (0.95)	14 (0.70)	19 (0.95)
1989	Six years	Favorable status	1601 (80.5)	1555 (78.2)	1551 (78.0)	1538 (77.3)	1553 (78.1)
		Unfavorable status	0 (0.00)	85 (4.27)	120 (6.03)	118 (5.93)	113 (5.68)
1987	Nine years	Favorable status	1342 (67.5)	1192 (60.0)	1219 (61.3)	1217 (61.2)	1212 (61.0)
		Unfavorable status	0 (0.00)	297 (14.9)	331(16.7)	323 (16.3)	339 (17.1)
5972	All three times combined	Favorable status	4768 (79.8)	4585 (76.8)	4586 (76.8)	4569 (76.5)	4581 (76.7)
		Unfavorable status	0 (0.00)	392 (6.56)	470 (7.87)	364 (6.10)	471 (7.89)
		Total	4768 (79.8)	4977 (83.3)	5056 (84.7)	4933 (82.6)	5052 (84.6)

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Legend to table 4

All-cause death. Correct predictions of favorable (alive) and unfavorable (not alive) status made at 3 years, at 6 years, and at 9 years following randomisation in the 2199 placebo patients from the CLARICOR trial. Four covariate scenarios were examined with Cox regression (see text of columns 4, 5, 6, and 8). For comparison with the results of column 6, column 7 shows the corresponding results when the accelerated failures model was used.

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(1) Number of predictions made	(2) Time at which prediction was made	(3) Correctly predicted patient status	(4) Data without covariates included Both models N (%)	(5) Data including Standard predictors as covariates Cox model N (%)	(6) Data including Standard predictors + advanced biochemical predictors as covariates Cox model N (%)	(7) Data including Standard predictors + advanced biochemical predictors as covariates Parametric model N (%)	(8) Data including Standard predictors + Log(OPG/ng/L), Log(hsTnT/ng/L), and log(proBNP/ng/L) as covariates Cox model N (%)
1996	Three years	Favorable status	1514 (75.9)	1471 (73.7)	1464 (73.3)	1479 (74.1)	1463 (73.3)
		Unfavorable status	0 (0)	51 (2.56)	77 (3.86)	57 (2.86)	76 (3.81)
1989	Six years	Favorable status	1144 (57.5)	935 (47.0)	920 (46.3)	916 (46.1)	925 (46.5)
		Unfavorable status	0 (0)	349 (17.5)	370 (18.6)	368 (18.5)	367 (18.5))
1987	Nine years	Favorable status	0 (0)	504 (25.4)	542 (27.3)	550 (27.7)	549 (27.6)
		Unfavorable status	1115 (56.1)	774 (39.0)	792 (39.9)	803 (40.4)	779 (39.2)
5972	All three times combined	Favorable status	2658 (44.5)	2910 (48.7)	2926 (49.0)	2945 (49.3)	2937 (49.2)
		Unfavorable status	1115 (18.7)	1174 (19.7)	1239 (20.7)	1228 (20.6)	1222 (20.5)
		Total	3773 (63.2)	4084 (68.4)	4165 (69.7))	4173 (69.9)	4159 (69.6)

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Legend to table 5

The composite outcome of AMI, UAP, CeVD, and all-cause death. Correct predictions of favorable (no outcome so far) and unfavorable status made at 3, 6 and 9 years. Cox model: four covariate scenarios as in Table 4; and parametric model (column 7) for comparison with column 6. Note that log (OPG) qualified for inclusion in column 8.

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STROBE Statement items 1 to 12

Title and abstract	Item no	Recommendation	
	1	(a) Design in title	See page 1 the term 'placebo receiving' implies that controls from a trial were used
		(b) Abstract: informative and balanced summary of what was done and found	See abstract methods and results sections p 2 and p 3
Introduction			
Background/rationale	2	Scientific background and rationale	See introduction first section on page 5
Objectives	3	objectives	See introduction last section page 5
Methods			
Study design	4	Key elements of study design	See first section on the patients in material page 5
Setting	5	Setting, location, relevant dates, period of recruitment, follow-up, and data collection	See first section on the patients in material page 5 and the two sections on predictors and on the outcomes pages 6 and 7
Participants	6	(a) Cohort study eligibility, selection, follow-up	See first section on page 6 and introduction page 4 second section
Variables	7	Outcomes, predictors	See section 'the outcomes' on page 7 and the section on predictors on page 6 and 7, and table 1
Data sources/measurement	8	Sources of data, methods of assessment	See section on the outcomes on page 7 and the section on predictors on page 6 and 7, and table 1 plus references to methods.
Bias	9	Addressing potential sources of bias	See page 9 second section from above. Assessment of the potential bias due to missing values.
Study size	10	How study size was arrived at	See Hansen S et al: the CLARICOR trial design. HeartDrug 2001; 1:14-9
Quantitative variables	11	How quantitative variables were handled	They were all handled as continuous variables except for PAP-A which was dichotomized into normal vs elevated values (see table 1 and page 7 line 3)
Statistical methods	12	Statistical methods	See 'statistical analysis'

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STROBE Statement items 1 to 12

			page 8
		Missing data	See item 9
		Loss to follow-up	See page 6 last line of first section

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STROBE Statement items 13 to 22

Results			
	Item no	Recommendation	
Participants	13	Flow diagram during enrolment, randomisation, and follow-up in original trial of 2006.	See BMJ 2006;332;22-27 (paper is enclosed)
Descriptive data	14	(a) Characteristics of study participants (b) Number of participants with missing data for each variable (c) Summary of follow-up time	(a) See table 1 (b) See table 1 (c) See page 9 line 3 to5
Outcome data	15	Number of outcome events	See page 9 line 11 to 14
Main results	16	(a) Hazard rates (b) Results of predictions	(a) See tables 2, 3 (b) See tables 4 and 5
Other analyses	17	interaction	See inferential impact of the newer biomarkers page 9 first 3 lines
Discussion			
Key results	18	Summary of key results	See discussion page 11 first section
limitations	19	(a) Positive bias due to development of model and test of model using same data (b) Methodology (c) Selection bias (d) Prognosis may be worse than at present time (e) Only questionnaire data were collected at randomisation	(a) See page 12 last two lines 6 to 13 (b) See section on methodology page 12 (c) See limitations page 13 first two sections (d) See last 6 lines on page 13 and first two at page 14 (e) See page 14 line 3 to 14
Interpretations	20		See last section of discussion page 14
Generalisability	21		See (a), (b), (c), and (d) item 19
Other information			
Funding	22		See the section on acknowledgements and section on funding, both on page 19

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BMJ Open

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

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Primary Subject Heading:	Cardiovascular medicine
Secondary Subject Heading:	Evidence based practice
Keywords:	biomarker, Cardiology < INTERNAL MEDICINE, Coronary heart disease < CARDIOLOGY

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Prognostic value of 12 novel cardiological biomarkers in stable coronary 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 CLARICOR trial at a random time in their disease trajectory.

Setting

Five Copenhagen University cardiology departments and a coordinating centre

Participants

2199 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

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S) were not associated (P ≥ 0.01) with the outcomes, not even as single predictors. Adding the remaining ten biomarkers (high-sensitive assay cardiac troponin T; neutrophil gelatinase-associated lipocalin; osteoprotegerin; N-terminal pro-B-type natriuretic peptide; tumor necrosis factor receptor 1 and 2; PAPP-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 heart disease, the addition of 10 novel biomarkers to the prediction model improved the correct prediction of all-cause death and the composite outcome by less than 1.5%.

Trial registration ClinicalTrials.gov, NCT00121550. Date of registration 13 July 2005

Date of enrolment of first participant 12 October 1999

Keywords CLARICOR, cardiovascular disease, cardiovascular risk prediction, ischaemic heart disease, predictors, mortality.

Strengths and limitations of this study

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

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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 cardiovascular 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 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

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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) sTNFR1 and (10) 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 cardiovascular diseases.

All of these have been claimed to add some prognostic information in patients with stable coronary artery heart 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 acute myocardial infarction (AMI), unstable angina pectoris (UAP), cerebrovascular vascular disease (Ce-VD) and death.

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MATERIAL

The patients

The study population is the placebo patients from the CLARICOR-study.[3,4] Patients aged 18 to 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-days trial of clarithromycin versus placebo.[3,4] Out of the 4372 who were randomised during October 1999 through April 2000, 2201 were in the placebo group.

The main results of the trial were that clarithromycin increased the risk of cardiovascular 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 acute myocardial infarction or unstable angina pectoris within the previous 3 months; (2) had had intra-coronary interventions within the previous 6 months; (3) had impaired renal function; (4) had hepatic dysfunction; (5) had congestive heart failure (New York Heart Association (NYHA) 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.

15 of the 2201 participants were lost track of, due to emigration.

The predictors

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

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 – 80 degrees C. The quantities measured include lipoproteins,[16] high-sensitivity-C-reactive-protein/mg/L (hs-CRP/mg/L),[7] and glomerular filtration /rate/mL/min (GFR/mL/min) using creatinine.[17] Along with variables already mentioned, these quantities are those collectively referred to as 'standard predictors'.

Biomarkers included as newer biomarkers were YKL40/µg/L)[8]; high-sensitive assay cardiac troponin T/ng/L (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]; N-terminal pro-B-type natriuretic peptide/ng/L (proBNP/ng/L)[9]; cathepsin-B/µg/L[6,18]; endostatin/ng/mL[19]; cathepsin-S/µg/L[6,20]; soluble TNF receptor 1/pg/mL and soluble TNF receptor 2/pg/mL (sTNFR1/pg./mL and sTNFR2/pg/mL)[5,21]; neutrophil gelatinase-associated lipocalin/ng/L (NGAL/ng/L)[22]; calprotectin/mg/L[11]; and osteoprotegerin/ng/L (OPG/ng/L)[12]. Due to storage problems some marker data are missing on some patients.

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

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years +/- 3 months of follow-up. Last register follow-up was December 31, 2009. The public registers have an almost 100% coverage and the quality of these are 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: acute myocardial infarction (AMI), unstable angina pectoris (UAP), cerebrovascular disease (CeVD), or all-cause death.

METHODS

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 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 generalized gamma model of error.[28] A significance level of 0.01 was used to pinpoint

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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 hazard ratio is the factor by which the hazard increases when the logarithm increases by 1, i.e., when the concentration increases by a factor *e* = 2.72.

Biomarkers with a hazard **ratio** 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 percent 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]

Secondly, we report the areas under the ROCs (receiver operating characteristics), also known as AUCs or *C*-indices, obtained when the Cox-Breslow risk estimates are applied to the same time window 0-to-9 years. The conventional 'observed' AUCs summarizes a ROC plot of cumulative events against the cumulative event-free contingency, with cumulation from large to small estimated risks. The corresponding 'predicted' AUC is based on cumulating the predicted risks. AUCs represent the pairwise concordance rate between risks and outcomes. In order to reward correct prediction of time of event, we further determined a 'dynamic' *C*-index, alias risk concordance within any pair of participants whose event order is deducible from the 9-year data window.

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It is noted that in the ROC analysis it was not possible to add two time dependent covariates which were needed because both age and log(OPG/ng/L) violated the assumption of proportional hazard.

Ethics and safety

Ethics approval and consent to participate was given by VEKKF01-076/99; Danish Medicines Agency 2612-975; Danish Data Protection Agency 1999-1200-174; VEK H-B-2009-015.

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. The public involvement was trough the approvals given by the regional ethics committee (KF 01-076/99 and journal no. H-12012125), the Danish Medicines Agency (2612-975), and the Danish Data Protection Agency (1999-1200-174).

RESULTS

Table 1 Distributions of demographics, previous history, current medication, standard biochemical predictors, and newer biochemical predictors in 2201 placebo receiving

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patients from the CLARICOR trial. Abbreviations as in section on abbreviations in the main paper.

Quantity	Distribution
Demographics and previous history	
Sex (male) N (%)	1518 (69.0%)
Age/year mean (SD)	65.2 (10.4) 2199
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[a]	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 (Tri-glyceride/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 (proBNP/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 ≥ 4mIU/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

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FOOTNOTES

a) The value of N varies because the laboratory tests have missing values (mostly due to storage problems). log: natural logarithm.

Table 1 presents an overview of the covariates expected to be available from stable cardiovascular disease patients 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 2201 placebo patients, 1998 had complete biochemical data. As Little's test[29] had P = 0.49, suggesting that the values were missing completely at random, we used complete case analyses in the following. The composition of the two groups appears consistent.

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 supplementary file S1, tables S1 and S2). 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 table 2S in

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supplementary file S1). The latter equation now included the 'standard predictors', plus the remaining 10 newer biomarkers and the

above-mentioned time dependent covariates. It appears from supplementary table S2 that only log (proBNP/ng/L), log (hs-cTnT/ng/L), and log (OPG/ng/L) •time/year contributed significantly to the prediction.

Table 2 The two outcomes (1) all-cause death and (2) the composite outcome of AMI, UAP, CeVD, and all-cause death were studied. The results shown are the number and percentages of correct predictions (P of prediction =0.5 used as cut off) of favorable (no outcome so far) and unfavorable status (the outcome occurred during the interval) made at 3, 6 and 9 years. Using the Cox model three covariate scenarios were compared.

Model and covariates included in model	**Total number of predictions made per outcome**	**Number and percent of correct predictions of events**	
		All-cause death N (%)	**Composite of AMI[a] ,UAP[b], Ce-VD[c], and 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)

a) AMI acute myocardial infarction
b) UAP unstable angina pectoris
c) Ce-VD cerebrovascular disease

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Table 2 (see also supplementary file S1 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 percent 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.

Using the parametric model in place of the Cox model we obtained quite similar results (see tables S3 and S4 in supplementary file S1). The same was true if we only included log (proBNP/ng/L), log (hs-cTnT/ng/L), and log (OPG/ng/L) instead of all 10 biomarkers when the Cox model was used (see tables S3 and S4 in the supplementary file S1).

Table 3. *C*-indices. Cox model estimates applied to the 0-9-year follow-up window (*n* = 1998).

	Binary-outcome *C* (AUC), observed (predicted)	**Dynamic *C*,** observed
Composite outcome (1115 events)[a]		
Standard predictors (SP) only	0.711 (0.707)	0.640
The 10 newer markers & SP	0.732 (0.732)	0.657
Log(hsTnT/ng/L) + log(proBNP/ng/L) + SP	0.730 (0.730)	0.656

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All-cause death (644 deaths) SP only The 10 newer markers & SP Log(hsTnT/ng/L) + log(proBNP/ng/L) + SP	 0.792 (0.793) 0.824 (0.816) 0.821 (0.813)	 0.737 0.765 0.762

a) Composite outcome: first occurrence of acute myocardial infarction. unstable angina pectoris, cerebrovascular disease or death. SP: 'standard predictors,' see table 1.

Table 3 summarizes 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 again almost all the marker information is contained in log(hsTNT/ng/L) and log(proBNP/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.

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 P=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 heart disease, 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 less than 1.5%.

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Our patients resemble those of The Prospective Observational Longitudinal Registry of Patients with Stable Coronary Heart Disease (CLARIFY) study[30] which enrolled 20.291 patients. The CLARIFY patients had been observed with a median of 24.1 months. However, enrollment took place 10 years later than in the CLARICOR trial and the incidence of cardiovascular deaths or myocardial infarctions in these patients was considerably lower,[30] probably reflecting improved quality of treatment and more frequent statin treatment in the CLARIFY patients (84% compared to 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 use the same data that we used to develop the model. Clearly this is bound to produce overly optimistic results compared to 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 coronary heart disease are likely going to be worse than observed here.

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 would a sophisticated integral over continuous time.

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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 (0.1%), 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).

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.

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The patients recruited for the CLARICOR trial were diagnosed with coronary heart disease 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.[31] 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 stable coronary heart disease patients 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.[32]

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

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been shown to of modest help in evaluating cardiovascular risk assessment in asymptomatic people not suffering from CAD.[33]

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 novel biomarkers all except for osteoprotegerin could be replaced by hs-cTnT and proBNP.

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Abbreviations

AMI: acute myocardial infarct; Apo A1: apoprotein A1; Apo B: apoprotein B; CeVD: cerebro-vascular disease; Chol-HDL: cholesterol high density lipoprotein; Chol-LDL: cholesterol low density lipoprotein; CLARICOR: Clarithromycin for patients with stable coronary heart disease; CRP: c-reactive protein; GFR: Glomerular filtration rate; hs-cTnT: High-sensitive assay cardiac troponin T; NGAL: neutrophil gelatinase-associated lipocalin; NYHA: New York Heart Association; OPG: osteoprotegerin; PREMAC: Predictors for major cardiovascular outcomes in stable ischaemic heart disease; proBNP: N-terminal pro-B-type natriuretic peptide;

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TNFR1: tumor necrosis factor receptor 1; TNFR2: tumor necrosis factor receptor 2; UAP: unstable angina pectoris.

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Availability of data and materials

All pertinent anonymised data will be uploaded at ZENODO (http://zenodo.org/) when the individual manuscripts have been published.

Authors' contributions

PW, JH, JCJ, and CG contributed substantially to the concept and design and

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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, 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, CG contributed substantially to the interpretation of the data.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

1. Winkel P, Jakobsen JC, Hilden J, et al. Prognostic value of routinely available data in patients with stable coronary heart disease. A 10-year follow-up of patients sampled at random times during their disease course. Open Heart 2018:e000808. Doi:10.1136/openhrt-2018-000808

2. Winkel P, Jakobsen JC, Hilden J, Lange T, Jensen GB, Kjøller E, et al: Predictors for major cardiovascular outcomes in stable ischaemic heart disease (PREMAC): statistical analysis plan for data originating from the CLARICOR (clarithromycin for patients with stable coronary heart disease) trial: Diagnostic and Prognostic Research (2017) 1:10 DOI 10.1186/s41512-017-0009-y.

3. Jespersen CM, Als-Nielsen B, Damgaard M, Hansen JF, Hansen S, Helø OH, et al: Randomised placebo controlled multicentre trial to assess short term clarithromycin for patients with stable coronary heart disease: CLARICOR trial. BMJ. 2006; 332:22–7.

For peer review only

4. Hansen S, Als-Nielsen B, Damgaard M, Helø OH, Petersen L, Jespersen CM, et al: Intervention with clarithromycin in patients with stable coronary heart disease: the CLARICOR trial design. Heart Drug. 2001; 1:14–9.

5. Carlsson AC, Ruge T, Kjøller E, Hilden J, Kolmos HJ, Sajadieh A o. a. 10-year associations between tumor necrosis factor receptors 1 and 2 and cardiovascular events in patients with stable coronary heart disease: a CLARICOR (effect of clarithromycin on mortality and morbidity in patients with coronary heart disease) trial substudy. Journal of the American Heart Association 2018 apr 23;7(9): e 008299 Doi:10. 116/JAHA 127.008299

6. Wuopio J, Hilden J, Bring C, Kastrup J, Sajadieh A, Jensen GB o.a. Cathepsin B and S as markers for cardiovascular risk and all-cause mortality in patients with stable coronary heart disease during 10 years: a CLARICOR trial substudy. Atherosclerosis. 2018 nov 1:278: 97 to 102. Tilgængelig fra, DOI: 10. 1016/j atherosclerosis. 2018.09.006

7. Harutyunyan MJ, Mathiasen AB, Winkel P, Gøtze JP, Hansen JF, Hildebrandt P, et al: High-sensitivity C-reactive protein and N-terminal pro-B-type natriuretic peptide in patients with stable coronary heart disease: a prognostic study within the CLARICOR trial. Scand J Clin Lab Invest. 2011; 71:52–62.

8. Kastrup J, Johansen JS, Winkel P, Hansen JF, Hildebrandt P, Jensen GB, et al.High serum YKL40 concentration is associated with cardiovascular and all-cause mortality in patients with stable coronary artery disease. Eur. Heart J. 2009; 30:1066-72.

9. Lyngbaek S, Winkel P, Gotze JP, Kastrup J, Gluud C, Kolmos HJ, et al. Risk stratification in stable coronary artery disease is possible at cardiac troponin levels below conventional detection and is improved by use of N-terminal pro-B-type natriuretic peptide. European Journal of Preventive Cardiology 2014; 21:1275-1284.

For peer review only

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10. Iversen KK, Teisner B, Winkel P, Gluud C, Kjoller E, Kolmos HJ, et al. Pregnancy associated plasma protein-A as a marker for myocardial infarction and death in patients with stable coronary artery disease: a prognostic study within the CLARICOR Trial. Atherosclerosis 2011; 214:203-208.

11. Larsen SB, Grove EL, Pareek M, Kristensen SD, Hvas AM. Calprotectin and platelet aggregation in patients with stable coronary artery disease. PloS ONE 2015; 10(5):e0125992.

12. Bjerre M, Hilden J, Kastrup J, Skoog M, Hansen JF, Kolmos HJ, et al. Osteoprotegerin independently predicts mortality in patients with stable coronary artery disease: the CLARICOR trial. Scandinavian Journal of Clinical and Laboratory Investigation 2014; 74:657-664.

13. Winkel P, Hilden J, Hansen JF, Kastrup J, Kolmos HJ, Kjoller E, et al. Clarithromycin for stable coronary heart disease increases all-cause and cardiovascular mortality and cerebrovascular morbidity over 10years in the CLARICOR randomised, blinded clinical trial. International Journal of Cardiology 2015, 182:459-465.

14. Winkel P, Hilden J, Fischer Hansen J, Hildebrandt P, Kastrup J, Kolmos HJ, et al: Excess sudden cardiac deaths after short-term clarithromycin administration in the CLARICOR trial: why is this so, and why are statins protective? Cardiology. 2011; 118:63–67.

15. Gluud C, Als-Nielsen B, Damgaard M, Fischer Hansen J, Hansen S, Helø OH, et al: Clarithromycin for 2 weeks for stable coronary heart disease: 6-year follow-up of the

For peer review only

25

CLARICOR randomized trial and updated meta-analysis of antibiotics for coronary heart disease. Cardiology. 2008; 111:280

16. Helmersson-Karlqvist J, Ridefelt P, Lind L, Larsson A. Reference values for 34 frequently used laboratory tests in 80-year-old men and women. Maturitas. 2016; 92: 97-101.

17. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al: Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012;367:20–9.

18. Wang N, Bai X, Jin B, Han W, Sun X, Chen X. The association of serum cathepsin-B concentration with age-related cardiovascular-renal subclinical state in a healthy Chinese population. Arch Gerontol Geriatr. 2016; 65:146-55.

19. Arnlov J, Ruge T, Ingelsson E, Sundstrom J, Lind L. Serum endostatin and risk of mortality in the elderly: findings from 2 community-based cohorts. Arterioscler Thromb Vasc Biol.2013, 33: 2689-95.

20. Jobs E, Ingelsson E, Risérus U, Nerpin E, Jobs M, Sundström J, et al. Association between serum cathepsin S and mortality in older adults. JAMA. 2011; 306:1113-21.

21. Carlsson AC, Larsson TE, Helmersson-Karlqvist J, Larsson A, Lind L, Ärnlöv J. Soluble TNF receptors and kidney dysfunction in the elderly. J Am Soc Nephrol. 2014;25:1313-20.

22. Helmersson-Karlqvist J, Larsson A, Carlsson AC, Venge P, Sundstrom J, Ingelsson E, et al. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is associated

For peer review only

with mortality in a community-based cohort of older Swedish men. Atherosclerosis 2013; 227:408-13.

23. Kjøller E, Hilden J, Winkel P, Frandsen NJ, Galatius S, Jensen G, et al: Good interobserver agreement was attainable on outcome adjudication in patients with stable coronary heart disease. J Clin Epidemiol. 2012; 65:444–53.

24. Kjøller E, Hilden J, Winkel P, Galatius S, Frandsen NJ, Jensen GB, et al: Agreement between public register and adjudication committee outcome in a cardiovascular randomized clinical trial. Am Heart J. 2014; 168:197–204.ok

25. Helweg-Larsen K: The Danish register of causes of death. Scand J Public Health. 2011;39(7 30. Suppl):26-9.

26. Lynge E, Sandegaard JL, Rebolj M: The Danish National Patient Register. Scand J Public Health. 2011;39(7 suppl):30–3.

27. Lin DY, Wei LJ, Ying Z: Checking the Cox model with cumulative sums of martingale-based residuals. Biometrika. 1993; 80:557–72.

28. Allison PD: Survival Analysis Using SAS: A Practical Guide, second edition 2010 SAS institute Inc. Cary, NC, USA.

29. Little R: A test of missing completely at random for multivariate data with missing values. J Am Stat Assoc. 1988; 83:1198–1202.

30. Steg PG, Greenlaw N, Tendera M, et al. Prevalence of anginal symptoms and myocardial ischemia and their effect on clinical outcomes in outpatients with stable coronary

For peer review only

artery disease. Data from the International Observational CLARIFY Registry. JAMA Intern Med. 2014;174(10):1651-1659. doi:10.1001/jamainternmed.2014.3773

31. Schmidt M, Jacobsen JB, Lash TL, Bøtker HE, Sørensen HT: 25-year trends in first time hospitalisation for acute myocardial infarction, subsequent short and long-term mortality, and the prognostic impact of sex and comorbidity: a Danish nationwide cohort study. BMJ. 2012;344: e356.

32. Solomon SD, Claggett B,Desai AS, *et al*. Influence of ejection fraction on outcomes and efficacy of sacubitril/valsartan (lcz696) in heart failure with reduced ejection fraction. Circulation 2016;9:e00274.

33. Scott IA. Evaluating cardiovascular risk assessment in asymptomatic people.BMJ 2009:a2844doi:10.1136/bmj.a2844

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Supplementary file S1

Table 1S All-cause mortality hazard ratios (HR) of newer biochemical predictors not routinely used in clinical work when each of these predictors is used alone (columns 2 to 4), and when it is used in combination with the 'standard predictors'[a] (column 5 to 7). Two of them were then discarded and each of the remaining 10 was assessed when used in combination with the standard predictors and the remaining 9 of the 10 newer biochemical predictors selected among the 12 candidates (columns 8 to 10).

Newer biochemical candidate predictor	When candidate predictor is the only predictor included in the model (stratified by centre)			When 'standard predictors' is added to the model (stratified by centre)			When in addition the 10 selected predictors are added to the model (stratified by centre)		
	HR[b]	95% CI	P	HR	95% CI	P	HR	95% CI	P
log (endostatin/ng/mL)	3.49	2.81 to 4.33	<0.0001	1.75	1.34 to 2.27	<0.0001	1.23	0.92 to 1.63	0.16
log (OPG/ng/L)	3.37	2.88 to 3.94	<0.0001	1.68	1.35 to 2.09	<0.0001	1.21	0.97 to 1.63	0.092
log (sTNFR1/pg/mL)	3.80	3.19 to 4.54	<0.0001	1.84	1.46 to 2.33	<0.0001	1.10	0.81 to 1.48	0.55
og (sTNFR2/pg/mL)	5.45	4.40 to 6.76	<0.0001	2.39	1.80 to 3.18	<0.0001	1.43	0.99 to 2.07	0.056
log(proBNP/ng/L)	1.76	1.66 to 1.87	<0.0001	1.44	1.34 to 1.55	<0.0001	1.28	1.19 to 1.39	<0.0001
log(hs-cTnT/ng/L)	2.31	2.16 to 2.47	<0.0001	1.73	1.56 to 1.92	<0.0001	1.46	1.30 to 1.65	<0.0001
PAPP-A_binary[c]	1.84	1.53 to 2.21	<0.0001	1.39	1.15 to 1.68	0.0007	0.85	0.69 to 1.03	0.10
log (YKL40/µg/L)	1.76	1.59 to 1.95	<0.0001	1.32	1.17 to 1.49	<0.0001	1.10	0.97 to 1.25	0.15
log (NGAL/ng/L)	1.33	1.12 to 1.57	0.0011	1.03	0.85 to 1.24	0.78	0.90	0.74 to 1.10	0.30
log(Calprotectin/)	1.08	0.95 to 1.23	0.25	1.02	0.89 to 1.18	0.74	Not included in analysis		
log (Cathepsin-B/µg/L)	2.81	2.40 to 3.28	<0.0001	1.43	1.19 to 1.73	0.0002	1.09	0.89 to 1.33	0.42
log (Cathepsin-S/µg/L)	1.12	0.86 to 1.47	0.40	1.10	0.83 to 1.45	0.51	Not included in analysis		

a) The standard predictors are shown in Table 1.

b) Hazard ratio associated with unit increase on log scale, except for PAPP-A (binary).

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c) Binary quantity. 1: PAPP-A was ≥ 4 mIU/L, 0: PAPP-A was < 4 mIU/L.

1. Inferential impact of the newer biomarkers

As the proportional hazard's assumption was violated for age[29] and age interacted significantly with time since randomisation, we included an interaction between age at entry and time (since randomisation) in the inference analyses.

Table 1S shows the results of a Cox regression of all-cause death on each of the 12 biomarkers when the biomarker was used alone as a covariate (columns 2 through 4), and when it was used in combination with the 'standard predictors' (columns 5 through 7).

Columns 8 through 10 in Table 1S shows the result of a regression of the outcome on the 'standard predictors' and the 10 best biochemical predictors. Now only log (proBNP /ng/L) and log(hs-cTnT/ng/L) have a HR significantly (P < 0.01) different from 1. Log(calprotectin/mg/L) and log(cathepsin-S/µg/L) did not have an inferential impact (P < 0.01 not attained), not even when used alone.

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Table 2S the composite outcome (comprising first occurrence of acute myocardial infarction, unstable angina pectoris, cerebro-vascular disease, and death). Hazard ratios of each of 13 biochemical predictors not routinely used in clinical work when each of these predictors is used alone (columns 2 to 4), and when it is used in combination with the 'standard predictors' (columns 5 to 7). Two of them were then discarded and each of the remaining 11 was assessed when used in combination with the standard predictors and the remaining 10 of the 11 newer biochemical predictors selected among the 13 candidates (columns 8 to 10)

Newer biochemical candidate predictor	When candidate predictor is the only predictor included in the model (stratified by centre)			When 'standard predictors' is added to the model (stratified by centre)			When in addition the 11[a] selected predictors are added to the model (stratified by centre)		
	HR	95% CI of HR	P	HR	95% CI of HR	P	HR	95% CI of HR	P
log (Endostatin/ng/mL)	2.18	1.84 to 2.58	<0.0001	1.44	1.17 to 1.72	0.0006	1.23	0.99 to 1.54	0.062
log (OPG/ng/L)	1.34	1.05 to 1.71	0.019	0.94	0.70 to 1.26	0.67	0.78	0.58 to 1.04	0.094
log (OPG/ng/L) ·time/year[b]	1.11	1.06 to 1.16	<0.0001	1.09	1.03 to 1.16	0.0022	1.104	1.044 to 1.168	0.0005
log (sTNFR1/pg/mL)	2.14	1.86 to 2.46	<0.0001	1.33	1.11 to 1.60	0.0021	1.05	0.84 to 1.32	0.67
log (sTNFR2/pg/mL)	2.56	2.15 to 3.03	<0.0001	1.49	1.19 to 1.85	0.0004	1.13	0.85 to 1.50	0.40
log (proBNP/ng/L)	1.37	1.31 to 1.44	<0.0001	1.26	1.19 to 1.33	<0.0001	1.18	1.11 to 1.25	<0.0001
log (hs-cTnT/ng/L)	1.83	1.70 to 1.97	<0.0001	1.49	1.35 to 1.64	<0.0001	1.31	1.17 to 1.46	<0.0001
PaPP-A (binary)[c]	1.45	1.24 to 1.70	<0.0001	1.24	1.06 to 1.46	0.0077	0.89	0.75 to 1.05	0.15
log (YKL40/µg/L)	1.35	1.24 to 1.47	<0.0001	1.13	1.03 to 1.24	0.013	1.01	0.91 to 1.11	0.93
log (NGAL/ng/L)	1.23	1.08 to 1.40	0.0023	1.03	0.89 to 1.19	0.73	0.97	0.84 to 1.13	0.74
log (Calprotectin/)	1.06	0.95 to 1.17	0.32	1.00	0.90 to 1.12	0.95	Not included in analysis		
log (cathepsin-B/µg/L)	1.70	1.50 to 1.93	<0.0001	1.17	1.01 to 1.35	0.040	0.99	0.85 to 1.16	0.92
log (cathepsin-S/µg/L)	1.06	0.86 to 1.31	0.59	0.98	0.79 to 1.22	0.88	Not included in analysis		

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a) Note that now a time dependent covariate has been added [log (OPG/ng/L) · time/year] to the 10 original predictors.

b) log (OPG/ng/L) significantly violated the proportional hazard assumption. We found a significant linear relationship between log (OPG/ng/L) and time since randomisation which may explain the violation. The product of log (OPG/ng/L) and time/year was therefore included in the inference analysis. However, when the Cox model is used for prediction, time dependent covariates are not allowed (SAS 9.4). Therefore, in the latter context we only include log (OPG/ng/L).

c) Binary quantity. 1: PAPP-A was ≥4 mIU/L, 0: PAPP-A was <4 mIU/L.

Table 2S corresponds to Table 1S except that the outcome is the composite outcome. It is noted that a time-dependent covariate is now included because log (OPG/ng/L) violated the proportional hazard assumption. This was remedied by including the covariate log (OPG/ng/L) ·time/year. It is seen that when all the biomarkers were included in the Cox analysis log(OPG/ng/L)·time/year, log(proBNP/ng/L), and log(hs-cTnT/ng/L) were the only ones which had a P value below the threshold of 0.01. Again log(calprotectin/mg/L) and log(cathepsin-S/µg/L) could be excluded from the final analysis, the result of which is shown in columns 8 through 10.

2. Practical impact of the novel biomarkers

Table 3S All-cause death. Correct predictions of favorable (alive) and unfavorable (not alive) status made at 3 years, at 6 years, and at 9 years following randomisation in the 1998 placebo patients from the CLARICOR trial. Four covariate scenarios were examined with Cox regression (see text of columns 4, 5, 6, and 8). For comparison with the results of column 6, column 7 shows the corresponding results when the accelerated failures model was used.

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(1) Number of predictions made	(2) Time at which prediction was made	(3) Correctly predicted patient status	(4) Data without covariates included Both models N (%)	(5) Data including Standard predictors as covariates Cox model N (%)	(6) Data including Standard predictors + advanced biochemical predictors as covariates Cox model N (%)	(7) Data including Standard predictors + advanced biochemical predictors as covariates Parametric model N (%)	(8) Data including Standard predictors log(OPG/ng/L) log (hs-cTnT/ng/L) and log(proBNP/ng/L) as covariates Cox Model N (%)
1996	Three years	Favorable status	1825 (91.4)	1821 (91.2)	1816 (91.0)	1814 (90.9)	1816 (91.0)
		Unfavorable status	0 (0.00)	10 (0.50)	19 (0.95)	14 (0.70)	19 (0.95)
1989	Six years	Favorable status	1601 (80.5)	1555 (78.2)	1551 (78.0)	1538 (77.3)	1553 (78.1)
		Unfavorable status	0 (0.00)	85 (4.27)	120 (6.03)	118 (5.93)	113 (5.68)
1987	Nine years	Favorable status	1342 (67.5)	1192 (60.0)	1219 (61.3)	1217 (61.2)	1212 (61.0)
		Unfavorable status	0 (0.00)	297 (14.9)	331(16.7)	323 (16.3)	339 (17.1)
5972	All three times combined	Favorable status	4768 (79.8)	4585 (76.8)	4586 (76.8)	4569 (76.5)	4581 (76.7)
		Unfavorable status	0 (0.00)	392 (6.56)	470 (7.87)	364 (6.10)	471 (7.89)
		Total	4768 (79.8)	4977 (83.3)	5056 (84.7)	4933 (82.6)	5052 (84.6)

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The results of the predictions of survival status made at 3 years, at 6 years, and at 9 years following randomisation in the 1998 placebo patients are summarized in Table 3S.

When the 'standard predictors' were included as covariates (column 5) for all-cause mortality, 83.3% of the predictions were correct. Adding the 10 newer biochemical predictors (column 6) the percentage was increased by 1.4% to 84.7%.

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Figure 1S A

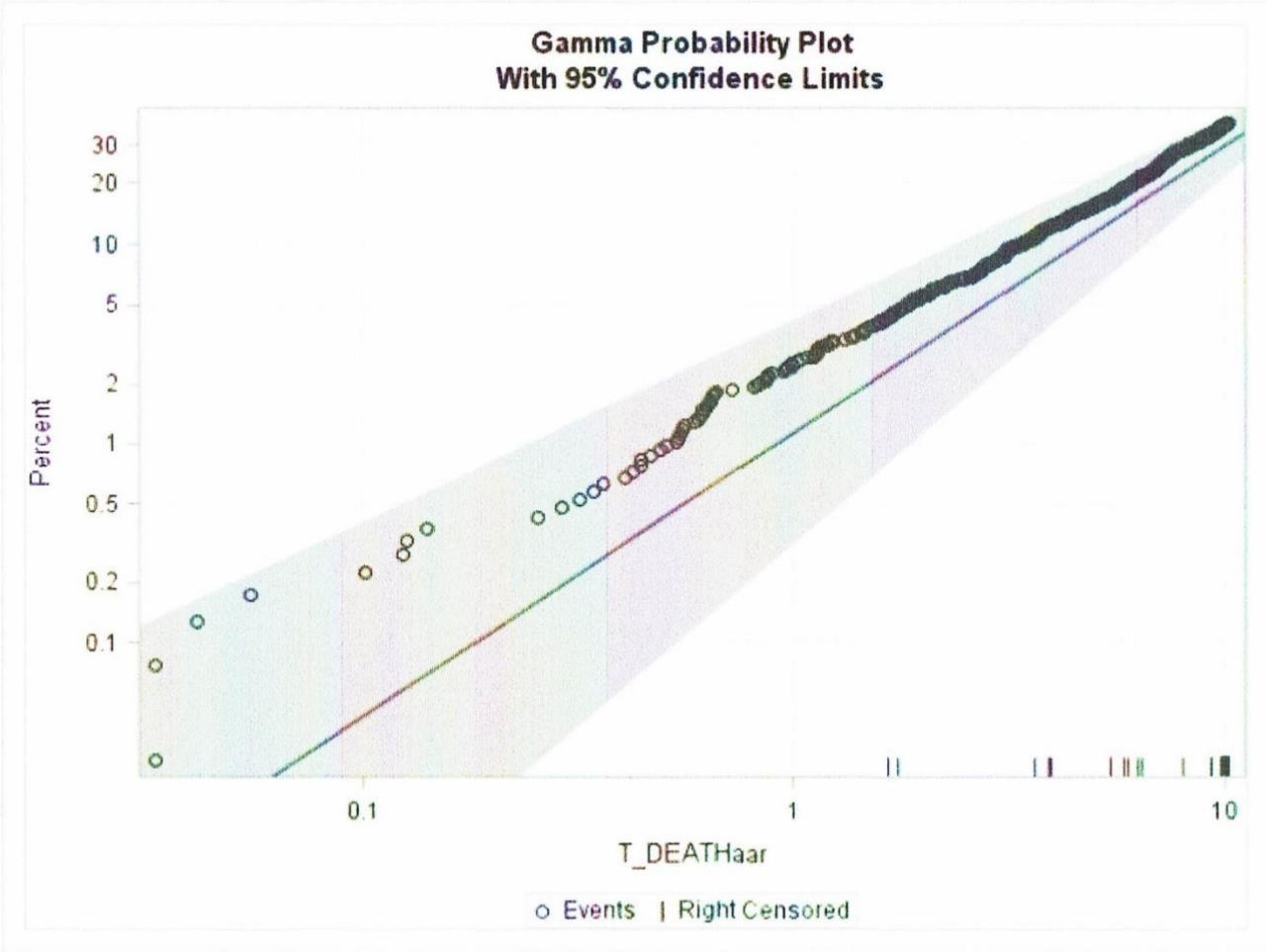

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Figure 1S B

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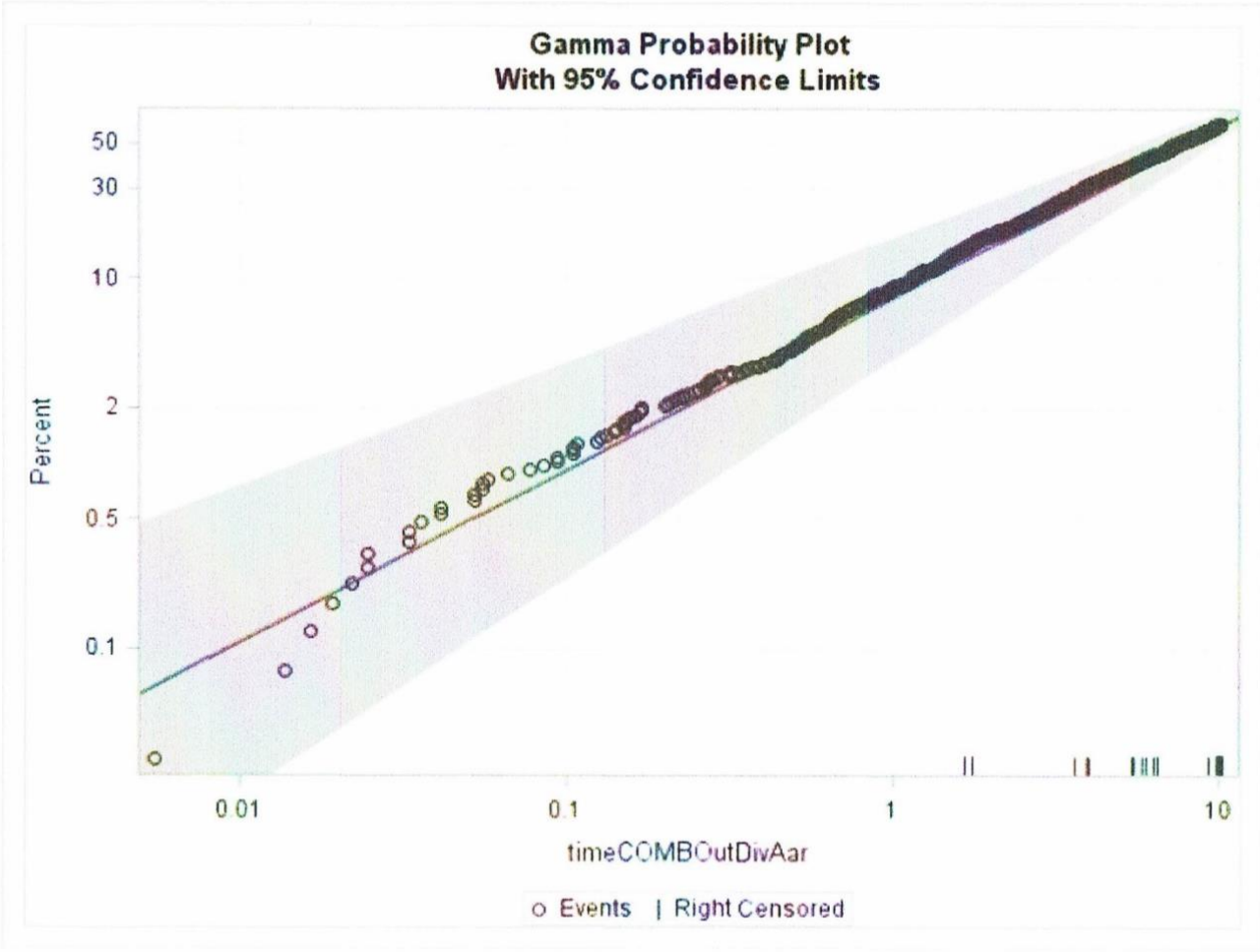

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The parametric model fitted the data reasonably well (see figure 1S A and B). The distribution of years to outcome using the accelerated failure model where the error term is modelled using the general gamma distribution showed that for both outcomes all values were within the 95% confidence limits. However, in case of all-cause death (see figure 1S A) the distribution was upwards biased but still within the 95% confidence limits.

It is noted that the results obtained with the parametric model (column 7 Tables S3 and S4) are not dramatically different from the corresponding results in column 6, when this theoretically equally valid model is used. When only the three significant predictors log(OPG/ng/L), (log (proBNP/ng/L), and log (hs-cTnT/ng/L)) were used in the Cox model in place of all 10 (column 8), the results were practically unaffected (compare columns 8 and 6).

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Table 4S the composite outcome of AMI, UAP, CeVD, and all-cause death. Correct predictions of favorable (no outcome so far) and unfavorable status made at 3, 6 and 9 years. Cox model: four covariate scenarios as in Table 4; and parametric model (column 7) for comparison with column 6. Note that log (OPG) qualified for inclusion in column 8.

(1) Number of predictions made	(2) Time at which prediction was made	(3) Correctly predicted patient status	(4) Data without covariates included Both models N (%)	(5) Data including Standard predictors as covariates Cox model N (%)	(6) Data including Standard predictors + advanced biochemical predictors as covariates Cox model N (%)	(7) Data including Standard predictors + advanced biochemical predictors as covariates Parametric model N (%)	(8) Data including Standard predictors + Log(OPG/ng/L), Log(hs-cTnT/ng/L), and log(proBNP/ng/L) as covariates Cox model N (%)
1996	Three years	Favorable status	1514 (75.9)	1471 (73.7)	1464 (73.3)	1479 (74.1)	1463 (73.3)
		Unfavorable status	0 (0)	51 (2.56)	77 (3.86)	57 (2.86)	76 (3.81)
1989	Six years	Favorable status	1144 (57.5)	935 (47.0)	920 (46.3)	916 (46.1)	925 (46.5)
		Unfavorable status	0 (0)	349 (17.5)	370 (18.6)	368 (18.5)	367 (18.5))
1987	Nine years	Favorable status	0 (0)	504 (25.4)	542 (27.3)	550 (27.7)	549 (27.6)
		Unfavorable status	1115 (56.1)	774 (39.0)	792 (39.9)	803 (40.4)	789 (39.2)
5972	All three times combined	Favorable status	2658 (44.5)	2910 (48.7)	2926 (49.0)	2945 (49.3)	2937 (49.2)
		Unfavorable status	1115 (18.7)	1174 (19.7)	1239 (20.7)	1228 (20.6)	1222 (20.5)
		Total	3773 (63.2)	4084 (68.4)	4165 (69.7))	4173 (69.9)	4159 (69.6)

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Table 4S shows the results corresponding to Table 3S obtained when the composite outcome was used. Including the 'standard predictors' in the model increases the percent correct predictions from 63.2 (see column 4, Table 4S) to 68.4 (see column 5, Table 4S), i.e. an increase of 5.2%. Adding the 10 newer biomarkers to the model increases the number of correct predictions by 1.3%. Using the parametric model does not change the results appreciably and neither does a reduction of the biomarkers to include only the three significant ones.

Legend to figure 1S

Figure 1S A Distribution of years to death using the accelerated failure model where the error term is modelled using the general gamma distribution.

Figure 1S B Distribution of years to composite outcome (AMI, UAP, CeVD, death) using the accelerated failure model where the error term is modelled using the general gamma distribution.

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STROBE Statement items 1 to 12

Title and abstract	Item no	Recommendation	
	1	(a) Design in title	See page 1 the term 'placebo receiving' implies that controls from a trial were used
		(b) Abstract: informative and balanced summary of what was done and found	See abstract methods and results sections p 2 and p 3
Introduction			
Background/rationale	2	Scientific background and rationale	See introduction first section on page 5
Objectives	3	objectives	See introduction last section page 5
Methods			
Study design	4	Key elements of study design	See first section on the patients in material page 5
Setting	5	Setting, location, relevant dates, period of recruitment, follow-up, and data collection	See first section on the patients in material page 5 and the two sections on predictors and on the outcomes pages 6 and 7
Participants	6	(a) Cohort study eligibility, selection, follow-up	See first section on page 6 and introduction page 4 second section
Variables	7	Outcomes, predictors	See section 'the outcomes' on page 7 and the section on predictors on page 6 and 7, and table 1
Data sources/measurement	8	Sources of data, methods of assessment	See section on the outcomes on page 7 and the section on predictors on page 6 and 7, and table 1 plus references to methods.
Bias	9	Addressing potential sources of bias	See page 9 second section from above. Assessment of the potential bias due to missing values.
Study size	10	How study size was arrived at	See Hansen S et al: the CLARICOR trial design. HeartDrug 2001; 1:14-9
Quantitative variables	11	How quantitative variables were handled	They were all handled as continuous variables except for PAP-A which was dichotomized into normal vs elevated values (see table 1 and page 7 line 3)
Statistical methods	12	Statistical methods	See 'statistical analysis'

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STROBE Statement items 1 to 12

			page 8
		Missing data	See item 9
		Loss to follow-up	See page 6 last line of first section

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STROBE Statement items 13 to 22

Results			
	Item no	Recommendation	
Participants	13	Flow diagram during enrolment, randomisation, and follow-up in original trial of 2006.	See BMJ 2006;332;22-27 (paper is enclosed)
Descriptive data	14	(a) Characteristics of study participants (b) Number of participants with missing data for each variable (c) Summary of follow-up time	(a) See table 1 (b) See table 1 (c) See page 9 line 3 to5
Outcome data	15	Number of outcome events	See page 9 line 11 to 14
Main results	16	(a) Hazard rates (b) Results of predictions	(a) See tables 2, 3 (b) See tables 4 and 5
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Discussion			
Key results	18	Summary of key results	See discussion page 11 first section
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Prognostic value of 12 novel cardiological biomarkers in stable coronary disease. A 10-year follow-up of the placebo group of the Copenhagen CLARICOR trial

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Prognostic value of 12 novel cardiological biomarkers in stable coronary 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 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

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S) were not associated (P ≥ 0.01) with the outcomes, not even as single predictors. Adding the remaining ten biomarkers (high-sensitive assay cardiac troponin T; neutrophil gelatinase-associated lipocalin; osteoprotegerin; N-terminal pro-B-type natriuretic peptide; tumor necrosis factor receptor 1 and 2; PAPP-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 heart disease, the addition of 10 novel biomarkers to the prediction model improved the correct prediction of all-cause death and the composite outcome by less than 1.5%.

Trial registration ClinicalTrials.gov, NCT00121550. Date of registration 13 July 2005

Date of enrolment of first participant 12 October 1999

Keywords CLARICOR, cardiovascular disease, cardiovascular risk prediction, ischaemic heart disease, predictors, mortality.

Strengths and limitations of this study

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

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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 cardiovascular 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 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

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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) sTNFR1 and (10) 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 cardiovascular diseases.

All of these have been claimed to add some prognostic information in patients with stable coronary artery heart 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 acute myocardial infarction (AMI), unstable angina pectoris (UAP), cerebrovascular vascular disease (Ce-VD) and death.

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MATERIAL

The patients

The study population is the placebo patients from the CLARICOR-study.[3,4] Patients aged 18 to 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-days 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 cardiovascular 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 acute myocardial infarction or unstable angina pectoris within the previous 3 months; (2) had had intra-coronary interventions within the previous 6 months; (3) had impaired renal function; (4) had hepatic dysfunction; (5) had congestive heart failure (New York Heart Association (NYHA) 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.

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The predictors

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

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 −80 degrees C. The quantities measured include lipoproteins,[16] high-sensitivity-C-reactive-protein/mg/L (hs-CRP/mg/L),[7] and glomerular filtration /rate/mL/min (GFR/mL/min) using creatinine.[17] Along with variables already mentioned, these quantities are those collectively referred to as 'standard predictors'.

Biomarkers included as newer biomarkers were YKL40/µg/L)[8]; high-sensitive assay cardiac troponin T/ng/L (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]; N-terminal pro-B-type natriuretic peptide/ng/L (proBNP/ng/L)[9]; cathepsin-B/µg/L[6,18]; endostatin/ng/mL[19]; cathepsin-S/µg/L[6,20]; soluble TNF receptor 1/pg/mL and soluble TNF receptor 2/pg/mL (sTNFR1/pg./mL and sTNFR2/pg/mL)[5,21]; neutrophil gelatinase-associated lipocalin/ng/L (NGAL/ng/L)[22]; calprotectin/mg/L[11]; and osteoprotegerin/ng/L (OPG/ng/L)[12]. Due to storage problems some marker data are missing on some patients.

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

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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 December 31, 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: acute myocardial infarction (AMI), unstable angina pectoris (UAP), cerebrovascular disease (CeVD), or all-cause death.

METHODS

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 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].

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We also analysed data using a parametric, accelerated failure-time model using the generalized 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 hazard ratio is the factor by which the hazard increases when the logarithm increases by 1, i.e., when the concentration increases by a factor e = 2.72.

Biomarkers with a hazard ratio with P value $\geq$ 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 percent 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]

Secondly, we report the areas under the ROCs (receiver operating characteristics), also known as 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

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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.

Ethics and safety

Ethics approval and consent to participate was given by VEKKF01-076/99; Danish Medicines Agency 2612-975; Danish Data Protection Agency 1999-1200-174; VEK H-B-2009-015.

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. The public involvement was trough the approvals given by the regional ethics committee (KF 01-076/99 and journal no. H-12012125), the Danish Medicines Agency (2612-975), and the Danish Data Protection Agency (1999-1200-174).

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RESULTS

Quantity	Distribution
Demographics and previous history	
Sex (male) N (%)	1518 (69.0%)
Age/year mean (SD)	65.2 (10.4) 2199
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[a]	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 (Tri-glyceride/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 (proBNP/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 ≥ 4mIU/L count (%) N	288 (13.1%) 2140
log (YKL40/µg/L) mean (SD) N	4.75 (0.66) 2163

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. Abbreviations as in section on abbreviations.

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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

FOOTNOTES

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a) The value of N varies because the laboratory tests have missing values (mostly due to storage problems). log: natural logarithm.

Table 1 presents an overview of the covariates expected to be available from stable cardiovascular disease patients 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. The composition of the two groups appears consistent.

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 supplementary file S1, 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) in the subsequent regression equation (see table 2S in supplementary file S1). The latter equation now included the 'standard predictors', plus the remaining 10 newer biomarkers and the above-mentioned time dependent covariates. It appears from

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supplementary table 2S that only log (proBNP/ng/L), log (hs-cTnT/ng/L), and log (OPG/ng/L) contributed significantly to the prediction.

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

Model and covariates included in model	**Total number of predictions made per outcome**	**Number and percent of correct predictions of events**	
		All-cause death N (%)	**Composite of AMI[a] ,UAP[b], Ce-VD[c], and 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)

a) AMI acute myocardial infarction
b) UAP unstable angina pectoris
c) Ce-VD cerebrovascular disease

Table 2 (see also supplementary file S1 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 percent 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

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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.

Using the parametric model in place of the Cox model we obtained quite similar results (see tables 3S and 4S in supplementary file S1 and figure 1S A-B). The same was true if we only included log (proBNP/ng/L), log (hs-cTnT/ng/L), and log (OPG/ng/L) instead of all 10 biomarkers when the Cox model was used (see tables 3S and 4S in the supplementary file S1).

Table 3. *C*-indices. Cox model estimates applied to the 0-9-year follow-up window (*n* = 1998).

	Binary-outcome *C* (AUC), observed (predicted)[a]	**Dynamic *C*,** Observed[b]
Composite outcome[c] (1115 events)		
Standard predictors (SP) only	0.711 (0.707)	0.640
The 10 newer markers & SP	0.732 (0.732)	0.657
Log(hsTnT/ng/L) + log(proBNP/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 & SP	0.824 (0.816)	0.765
Log(hsTnT/ng/L) + log(proBNP/ng/L) + SP	0.821 (0.813)	0.762

a) The 'observed' AUCs summarize 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.
b) Analogous concordance rate between time to event and predicted risk.
c) Composite outcome: first occurrence of acute myocardial infarction. unstable angina pectoris, cerebrovascular disease or death. SP 'standard predictors', see table1.

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Table 3 summarizes 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(hsTNT/ng/L), and log(proBNP/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.

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 heart disease, 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 less than 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, enrollment took place 10 years later than in the CLARICOR trial and the incidence of cardiovascular 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 to only 41% in the CLARICOR material). So, the age of our material is a weakness.

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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 to 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 coronary heart disease are likely going to be worse than observed here.

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 would a sophisticated integral over continuous time.

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).

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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 coronary heart disease 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 stable coronary heart disease patients as here defined include the fact that only questionnaire data

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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 cardiovascular risk assessment in asymptomatic people not suffering from CAD.[35]

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 proBNP, possibly supplemented by osteoprotegerin.

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Abbreviations

AMI: acute myocardial infarct; Apo A1: apoprotein A1; Apo B: apoprotein B; CeVD: cerebro-vascular disease; Chol-HDL: cholesterol high density lipoprotein; Chol-LDL: cholesterol low density lipoprotein; CLARICOR: Clarithromycin for patients with stable coronary heart disease; CRP: c-reactive protein; GFR: Glomerular filtration rate; hs-cTnT: High-sensitive assay cardiac troponin T; NGAL: neutrophil gelatinase-associated lipocalin; NYHA: New York Heart Association; OPG: osteoprotegerin; PREMAC: Predictors for major cardiovascular outcomes in stable ischaemic heart disease; proBNP: N-terminal pro-B-type natriuretic peptide;

TNFR1: tumor necrosis factor receptor 1; TNFR2: tumor necrosis factor receptor 2; UAP: unstable angina pectoris.

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Availability of data and materials

All pertinent anonymised data will be uploaded at ZENODO (http://zenodo.org/) when the individual manuscripts have been published.

Authors' contributions

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, 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

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resolved. PW, JCJ, JH, GBJ, EK, AS, JK, HJK, KKI, MB, AL, JÄ, CG contributed substantially to the interpretation of the data.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

1. Winkel P, Jakobsen JC, Hilden J, et al. Prognostic value of routinely available data in patients with stable coronary heart disease. A 10-year follow-up of patients sampled at random times during their disease course. Open Heart 2018:e000808. Doi:10.1136/openhrt-2018-000808

2. Winkel P, Jakobsen JC, Hilden J, Lange T, Jensen GB, Kjøller E, et al: Predictors for major cardiovascular outcomes in stable ischaemic heart disease (PREMAC): statistical analysis plan for data originating from the CLARICOR (clarithromycin for patients with stable coronary heart disease) trial: Diagnostic and Prognostic Research (2017) 1:10 DOI 10.1186/s41512-017-0009-y.

3. Jespersen CM, Als-Nielsen B, Damgaard M, Hansen JF, Hansen S, Helø OH, et al: Randomised placebo controlled multicentre trial to assess short term clarithromycin for patients with stable coronary heart disease: CLARICOR trial. BMJ. 2006; 332:22–7.

4. Hansen S, Als-Nielsen B, Damgaard M, Helø OH, Petersen L, Jespersen CM, et al: Intervention with clarithromycin in patients with stable coronary heart disease: the CLARICOR trial design. Heart Drug. 2001; 1:14–9.

5. Carlsson AC, Ruge T, Kjøller E, Hilden J, Kolmos HJ, Sajadieh A o. a. 10-year associations between tumor necrosis factor receptors 1 and 2 and cardiovascular events in patients with stable coronary heart disease: a CLARICOR (effect of clarithromycin on

For peer review only

mortality and morbidity in patients with coronary heart disease) trial substudy. Journal of the American Heart Association 2018 apr 23;7(9): e 008299 Doi:10. 116/JAHA 127.008299

6. Wuopio J, Hilden J, Bring C, Kastrup J, Sajadieh A, Jensen GB o.a. Cathepsin B and S as markers for cardiovascular risk and all-cause mortality in patients with stable coronary heart disease during 10 years: a CLARICOR trial substudy. Atherosclerosis. 2018 nov 1:278: 97 to 102. Tilgængelig fra, DOI: 10. 1016/j atherosclerosis. 2018.09.006

7. Harutyunyan MJ, Mathiasen AB, Winkel P, Gøtze JP, Hansen JF, Hildebrandt P, et al: High-sensitivity C-reactive protein and N-terminal pro-B-type natriuretic peptide in patients with stable coronary heart disease: a prognostic study within the CLARICOR trial. Scand J Clin Lab Invest. 2011; 71:52–62.

8. Schroder J, Jakobsen CJ, Winkel P, Hilden J, Jensen GB, Sajadieh A, et al. Prognosis and Reclassification by YKL-40 in Stable Coronary Artery Disease. J Am Heart Assoc. 2020;9: e014634. DOI: 10.1161/JAHA.119.014634.

9. Lyngbaek S, Winkel P, Gotze JP, Kastrup J, Gluud C, Kolmos HJ, et al. Risk stratification in stable coronary artery disease is possible at cardiac troponin levels below conventional detection and is improved by use of N-terminal pro-B-type natriuretic peptide. European Journal of Preventive Cardiology 2014; 21:1275-1284.

10. Iversen KK, Teisner B, Winkel P, Gluud C, Kjoller E, Kolmos HJ, et al. Pregnancy associated plasma protein-A as a marker for myocardial infarction and death in patients with stable coronary artery disease: a prognostic study within the CLARICOR Trial. Atherosclerosis 2011; 214:203-208.

For peer review only

25

11. Larsen SB, Grove EL, Pareek M, Kristensen SD, Hvas AM. Calprotectin and platelet aggregation in patients with stable coronary artery disease. PloS ONE 2015; 10(5):e0125992.

12. Bjerre M, Hilden J, Kastrup J, Skoog M, Hansen JF, Kolmos HJ, et al. Osteoprotegerin independently predicts mortality in patients with stable coronary artery disease: the CLARICOR trial. Scandinavian Journal of Clinical and Laboratory Investigation 2014; 74:657-664.

13. Winkel P, Hilden J, Hansen JF, Kastrup J, Kolmos HJ, Kjoller E, et al. Clarithromycin for stable coronary heart disease increases all-cause and cardiovascular mortality and cerebrovascular morbidity over 10years in the CLARICOR randomised, blinded clinical trial. International Journal of Cardiology 2015, 182:459-465.

14. Winkel P, Hilden J, Fischer Hansen J, Hildebrandt P, Kastrup J, Kolmos HJ, et al: Excess sudden cardiac deaths after short-term clarithromycin administration in the CLARICOR trial: why is this so, and why are statins protective? Cardiology. 2011; 118:63–67.

15. Gluud C, Als-Nielsen B, Damgaard M, Fischer Hansen J, Hansen S, Helø OH, et al: Clarithromycin for 2 weeks for stable coronary heart disease: 6-year follow-up of the CLARICOR randomized trial and updated meta-analysis of antibiotics for coronary heart disease. Cardiology. 2008; 111:280

For peer review only

16. Helmersson-Karlqvist J, Ridefelt P, Lind L, Larsson A. Reference values for 34 frequently used laboratory tests in 80-year-old men and women. Maturitas. 2016; 92: 97-101.

17. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al: Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012;367:20–9.

18. Wang N, Bai X, Jin B, Han W, Sun X, Chen X. The association of serum cathepsin-B concentration with age-related cardiovascular-renal subclinical state in a healthy Chinese population. Arch Gerontol Geriatr. 2016; 65:146-55.

19. Arnlov J, Ruge T, Ingelsson E, Sundstrom J, Lind L. Serum endostatin and risk of mortality in the elderly: findings from 2 community-based cohorts. Arterioscler Thromb Vasc Biol.2013, 33: 2689-95.

20. Jobs E, Ingelsson E, Risérus U, Nerpin E, Jobs M, Sundström J, et al. Association between serum cathepsin S and mortality in older adults. JAMA. 2011; 306:1113-21.

21. Carlsson AC, Larsson TE, Helmersson-Karlqvist J, Larsson A, Lind L, Ärnlöv J. Soluble TNF receptors and kidney dysfunction in the elderly. J Am Soc Nephrol. 2014;25:1313-20.

22. Helmersson-Karlqvist J, Larsson A, Carlsson AC, Venge P, Sundstrom J, Ingelsson E, et al. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is associated with mortality in a community-based cohort of older Swedish men. Atherosclerosis 2013; 227:408-13.

For peer review only

23. Kjøller E, Hilden J, Winkel P, Frandsen NJ, Galatius S, Jensen G, et al: Good interobserver agreement was attainable on outcome adjudication in patients with stable coronary heart disease. J Clin Epidemiol. 2012; 65:444–53.

24. Kjøller E, Hilden J, Winkel P, Galatius S, Frandsen NJ, Jensen GB, et al: Agreement between public register and adjudication committee outcome in a cardiovascular randomized clinical trial. Am Heart J. 2014; 168:197–204.ok

25. Helweg-Larsen K: The Danish register of causes of death. Scand J Public Health. 2011;39(7 30. Suppl):26-9.

26. Lynge E, Sandegaard JL, Rebolj M: The Danish National Patient Register. Scand J Public Health. 2011;39(7 suppl):30–3.

27. Lin DY, Wei LJ, Ying Z: Checking the Cox model with cumulative sums of martingale-based residuals. Biometrika. 1993; 80:557–72.

28. Allison PD: Survival Analysis Using SAS: A Practical Guide, second edition 2010 SAS institute Inc. Cary, NC, USA.

29. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med. 1996;15: 361-87.

For peer review only

28

30. Pencina MJ, d'Agostino RB. Overall C as a measure of discrimination in survival analysis: model specific population value and confidence interval estimation. Stat Med. 2004; 23: 2109 -23.

31. Little R: A test of missing completely at random for multivariate data with missing values. J Am Stat Assoc. 1988; 83:1198–1202.

32. Steg PG, Greenlaw N, Tendera M, et al. Prevalence of anginal symptoms and myocardial ischemia and their effect on clinical outcomes in outpatients with stable coronary artery disease. Data from the International Observational CLARIFY Registry. JAMA Intern Med. 2014;174(10):1651-1659. doi:10.1001/jamainternmed.2014.3773

33. Schmidt M, Jacobsen JB, Lash TL, Bøtker HE, Sørensen HT: 25-year trends in first time hospitalisation for acute myocardial infarction, subsequent short and long-term mortality, and the prognostic impact of sex and comorbidity: a Danish nationwide cohort study. BMJ. 2012;344: e356.

34. Solomon SD, Claggett B,Desai AS, *et al*. Influence of ejection fraction on outcomes and efficacy of sacubitril/valsartan (lcz696) in heart failure with reduced ejection fraction. Circulation 2016;9:e00274.

35. Scott IA. Evaluating cardiovascular risk assessment in asymptomatic people.BMJ 2009:a2844doi:10.1136/bmj.a2844

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Supplementary file S1

Table 1S All-cause mortality hazard ratios (HR) of newer biochemical predictors not routinely used in clinical work when each of these predictors is used alone (columns 2 to 4), and when it is used in combination with the 'standard predictors'[a] (column 5 to 7). Two of them were then discarded and each of the remaining 10 was assessed when used in combination with the standard predictors and the remaining 9 of the 10 newer biochemical predictors selected among the 12 candidates (columns 8 to 10).

Newer biochemical candidate predictor	When candidate predictor is the only predictor included in the model (stratified by centre)			When 'standard predictors' is added to the model (stratified by centre)			When in addition the 10 selected predictors are added to the model (stratified by centre)		
	HR[b]	95% CI	P	HR	95% CI	P	HR	95% CI	P
log (endostatin/ng/mL)	3.49	2.81 to 4.33	<0.0001	1.75	1.34 to 2.27	<0.0001	1.23	0.92 to 1.63	0.16
log (OPG/ng/L)	3.37	2.88 to 3.94	<0.0001	1.68	1.35 to 2.09	<0.0001	1.21	0.97 to 1.63	0.092
log (sTNFR1/pg/mL)	3.80	3.19 to 4.54	<0.0001	1.84	1.46 to 2.33	<0.0001	1.10	0.81 to 1.48	0.55
og (sTNFR2/pg/mL)	5.45	4.40 to 6.76	<0.0001	2.39	1.80 to 3.18	<0.0001	1.43	0.99 to 2.07	0.056
log(proBNP/ng/L)	1.76	1.66 to 1.87	<0.0001	1.44	1.34 to 1.55	<0.0001	1.28	1.19 to 1.39	<0.0001
log(hs-cTnT/ng/L)	2.31	2.16 to 2.47	<0.0001	1.73	1.56 to 1.92	<0.0001	1.46	1.30 to 1.65	<0.0001
PAPP-A_binary[c]	1.84	1.53 to 2.21	<0.0001	1.39	1.15 to 1.68	0.0007	0.85	0.69 to 1.03	0.10
log (YKL40/µg/L)	1.76	1.59 to 1.95	<0.0001	1.32	1.17 to 1.49	<0.0001	1.10	0.97 to 1.25	0.15
log (NGAL/ng/L)	1.33	1.12 to 1.57	0.0011	1.03	0.85 to 1.24	0.78	0.90	0.74 to 1.10	0.30
log(Calprotectin/)	1.08	0.95 to 1.23	0.25	1.02	0.89 to 1.18	0.74	Not included in analysis		
log (Cathepsin-B/µg/L)	2.81	2.40 to 3.28	<0.0001	1.43	1.19 to 1.73	0.0002	1.09	0.89 to 1.33	0.42
log (Cathepsin-S/µg/L)	1.12	0.86 to 1.47	0.40	1.10	0.83 to 1.45	0.51	Not included in analysis		

a) The standard predictors are shown in Table 1.

b) Hazard ratio associated with unit increase on log scale, except for PAPP-A (binary).

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c) Binary quantity. 1: PAPP-A was ≥ 4 mIU/L, 0: PAPP-A was < 4 mIU/L.

1. Inferential impact of the newer biomarkers

As the proportional hazard's assumption was violated for age[29] and age interacted significantly with time since randomisation, we included an interaction between age at entry and time (since randomisation) in the inference analyses.

Table 1S shows the results of a Cox regression of all-cause death on each of the 12 biomarkers when the biomarker was used alone as a covariate (columns 2 through 4), and when it was used in combination with the 'standard predictors' (columns 5 through 7).

Columns 8 through 10 in Table 1S shows the result of a regression of the outcome on the 'standard predictors' and the 10 best biochemical predictors. Now only log (proBNP /ng/L) and log(hs-cTnT/ng/L) have a HR significantly (P < 0.01) different from 1. Log(calprotectin/mg/L) and log(cathepsin-S/µg/L) did not have an inferential impact (P < 0.01 not attained), not even when used alone.

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Table 2S the composite outcome (comprising first occurrence of acute myocardial infarction, unstable angina pectoris, cerebro-vascular disease, and death). Hazard ratios of each of 13 biochemical predictors not routinely used in clinical work when each of these predictors is used alone (columns 2 to 4), and when it is used in combination with the 'standard predictors' (columns 5 to 7). Two of them were then discarded and each of the remaining 11 was assessed when used in combination with the standard predictors and the remaining 10 of the 11 newer biochemical predictors selected among the 13 candidates (columns 8 to 10)

Newer biochemical candidate predictor	When candidate predictor is the only predictor included in the model (stratified by centre)			When 'standard predictors' is added to the model (stratified by centre)			When in addition the 11[a] selected predictors are added to the model (stratified by centre)		
	HR	95% CI of HR	P	HR	95% CI of HR	P	HR	95% CI of HR	P
log (Endostatin/ng/mL)	2.18	1.84 to 2.58	<0.0001	1.44	1.17 to 1.72	0.0006	1.23	0.99 to 1.54	0.062
log (OPG/ng/L)	1.34	1.05 to 1.71	0.019	0.94	0.70 to 1.26	0.67	0.78	0.58 to 1.04	0.094
log (OPG/ng/L) ·time/year[b]	1.11	1.06 to 1.16	<0.0001	1.09	1.03 to 1.16	0.0022	1.104	1.044 to 1.168	0.0005
log (sTNFR1/pg/mL)	2.14	1.86 to 2.46	<0.0001	1.33	1.11 to 1.60	0.0021	1.05	0.84 to 1.32	0.67
log (sTNFR2/pg/mL)	2.56	2.15 to 3.03	<0.0001	1.49	1.19 to 1.85	0.0004	1.13	0.85 to 1.50	0.40
log (proBNP/ng/L)	1.37	1.31 to 1.44	<0.0001	1.26	1.19 to 1.33	<0.0001	1.18	1.11 to 1.25	<0.0001
log (hs-cTnT/ng/L)	1.83	1.70 to 1.97	<0.0001	1.49	1.35 to 1.64	<0.0001	1.31	1.17 to 1.46	<0.0001
PaPP-A (binary)[c]	1.45	1.24 to 1.70	<0.0001	1.24	1.06 to 1.46	0.0077	0.89	0.75 to 1.05	0.15
log (YKL40/µg/L)	1.35	1.24 to 1.47	<0.0001	1.13	1.03 to 1.24	0.013	1.01	0.91 to 1.11	0.93
log (NGAL/ng/L)	1.23	1.08 to 1.40	0.0023	1.03	0.89 to 1.19	0.73	0.97	0.84 to 1.13	0.74
log (Calprotectin/)	1.06	0.95 to 1.17	0.32	1.00	0.90 to 1.12	0.95	Not included in analysis		
log (cathepsin-B/µg/L)	1.70	1.50 to 1.93	<0.0001	1.17	1.01 to 1.35	0.040	0.99	0.85 to 1.16	0.92
log (cathepsin-S/µg/L)	1.06	0.86 to 1.31	0.59	0.98	0.79 to 1.22	0.88	Not included in analysis		

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a) Note that now a time dependent covariate has been added [log (OPG/ng/L) · time/year] to the 10 original predictors.

b) log (OPG/ng/L) significantly violated the proportional hazard assumption. We found a significant linear relationship between log (OPG/ng/L) and time since randomisation which may explain the violation. The product of log (OPG/ng/L) and time/year was therefore included in the inference analysis. However, when the Cox model is used for prediction, time dependent covariates are not allowed (SAS 9.4). Therefore, in the latter context we only include log (OPG/ng/L).

c) Binary quantity. 1: PAPP-A was ≥4 mIU/L, 0: PAPP-A was <4 mIU/L.

Table 2S corresponds to Table 1S except that the outcome is the composite outcome. It is noted that a time-dependent covariate is now included because log (OPG/ng/L) violated the proportional hazard assumption. This was remedied by including the covariate log (OPG/ng/L) ·time/year. It is seen that when all the biomarkers were included in the Cox analysis log(OPG/ng/L)·time/year, log(proBNP/ng/L), and log(hs-cTnT/ng/L) were the only ones which had a P value below the threshold of 0.01. Again log(calprotectin/mg/L) and log(cathepsin-S/µg/L) could be excluded from the final analysis, the result of which is shown in columns 8 through 10.

2. Practical impact of the novel biomarkers

Table 3S All-cause death. Correct predictions of favorable (alive) and unfavorable (not alive) status made at 3 years, at 6 years, and at 9 years following randomisation in the 1998 placebo patients from the CLARICOR trial. Four covariate scenarios were examined with Cox regression (see text of columns 4, 5, 6, and 8). For comparison with the results of column 6, column 7 shows the corresponding results when the accelerated failures model was used.

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(1) Number of predictions made	(2) Time at which prediction was made	(3) Correctly predicted patient status	(4) Data without covariates included Both models N (%)	(5) Data including Standard predictors as covariates Cox model N (%)	(6) Data including Standard predictors + advanced biochemical predictors as covariates Cox model N (%)	(7) Data including Standard predictors + advanced biochemical predictors as covariates Parametric model N (%)	(8) Data including Standard predictors log(OPG/ng/L) log (hs-cTnT/ng/L) and log(proBNP/ng/L) as covariates Cox Model N (%)
1996	Three years	Favorable status	1825 (91.4)	1821 (91.2)	1816 (91.0)	1814 (90.9)	1816 (91.0)
		Unfavorable status	0 (0.00)	10 (0.50)	19 (0.95)	14 (0.70)	19 (0.95)
1989	Six years	Favorable status	1601 (80.5)	1555 (78.2)	1551 (78.0)	1538 (77.3)	1553 (78.1)
		Unfavorable status	0 (0.00)	85 (4.27)	120 (6.03)	118 (5.93)	113 (5.68)
1987	Nine years	Favorable status	1342 (67.5)	1192 (60.0)	1219 (61.3)	1217 (61.2)	1212 (61.0)
		Unfavorable status	0 (0.00)	297 (14.9)	331(16.7)	323 (16.3)	339 (17.1)
5972	All three times combined	Favorable status	4768 (79.8)	4585 (76.8)	4586 (76.8)	4569 (76.5)	4581 (76.7)
		Unfavorable status	0 (0.00)	392 (6.56)	470 (7.87)	364 (6.10)	471 (7.89)
		Total	4768 (79.8)	4977 (83.3)	5056 (84.7)	4933 (82.6)	5052 (84.6)

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The results of the predictions of survival status made at 3 years, at 6 years, and at 9 years following randomisation in the 1998 placebo patients are summarized in Table 3S.

When the 'standard predictors' were included as covariates (column 5) for all-cause mortality, 83.3% of the predictions were correct. Adding the 10 newer biochemical predictors (column 6) the percentage was increased by 1.4% to 84.7%.

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Figure 1S A

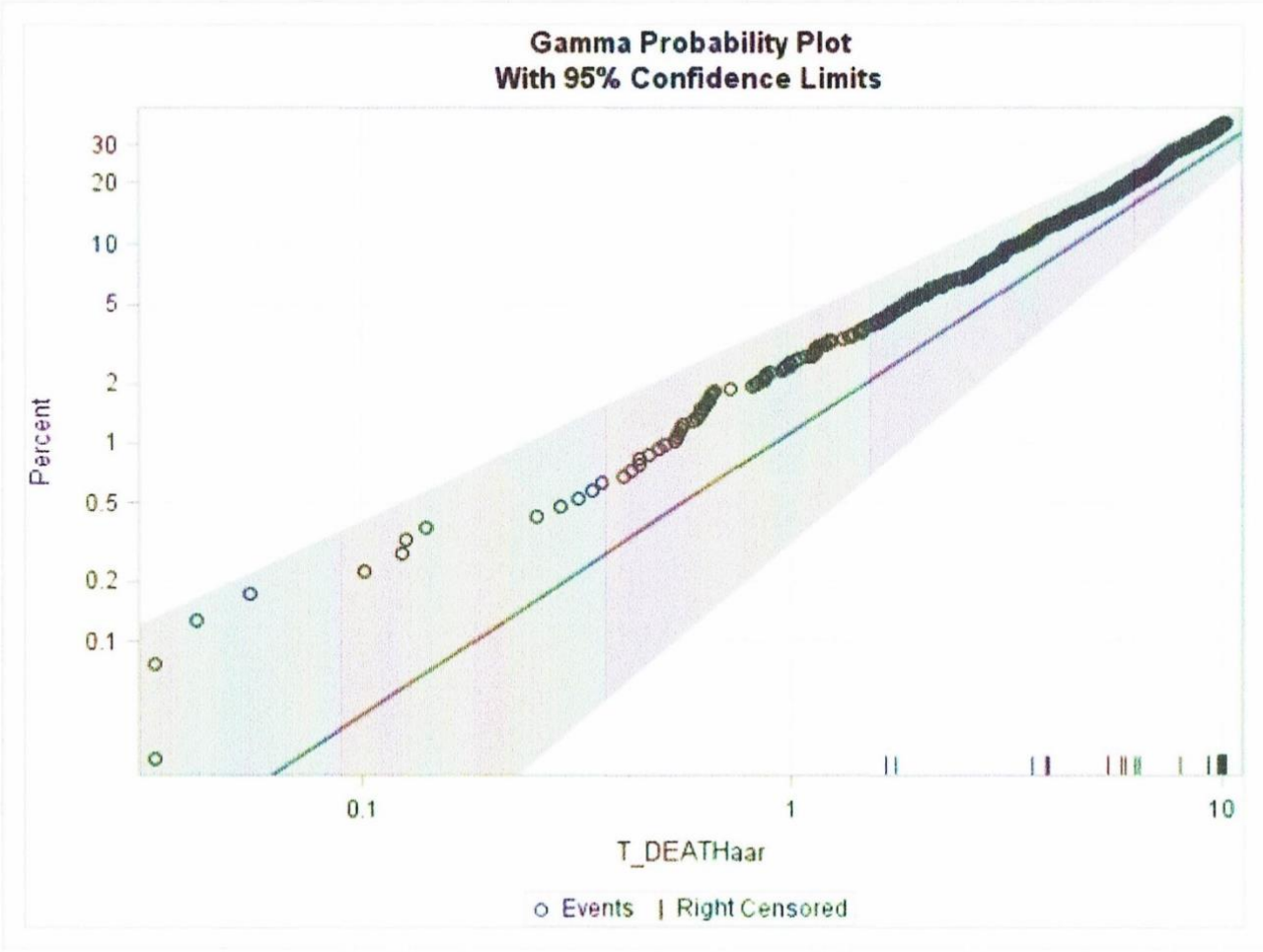

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Figure 1S B

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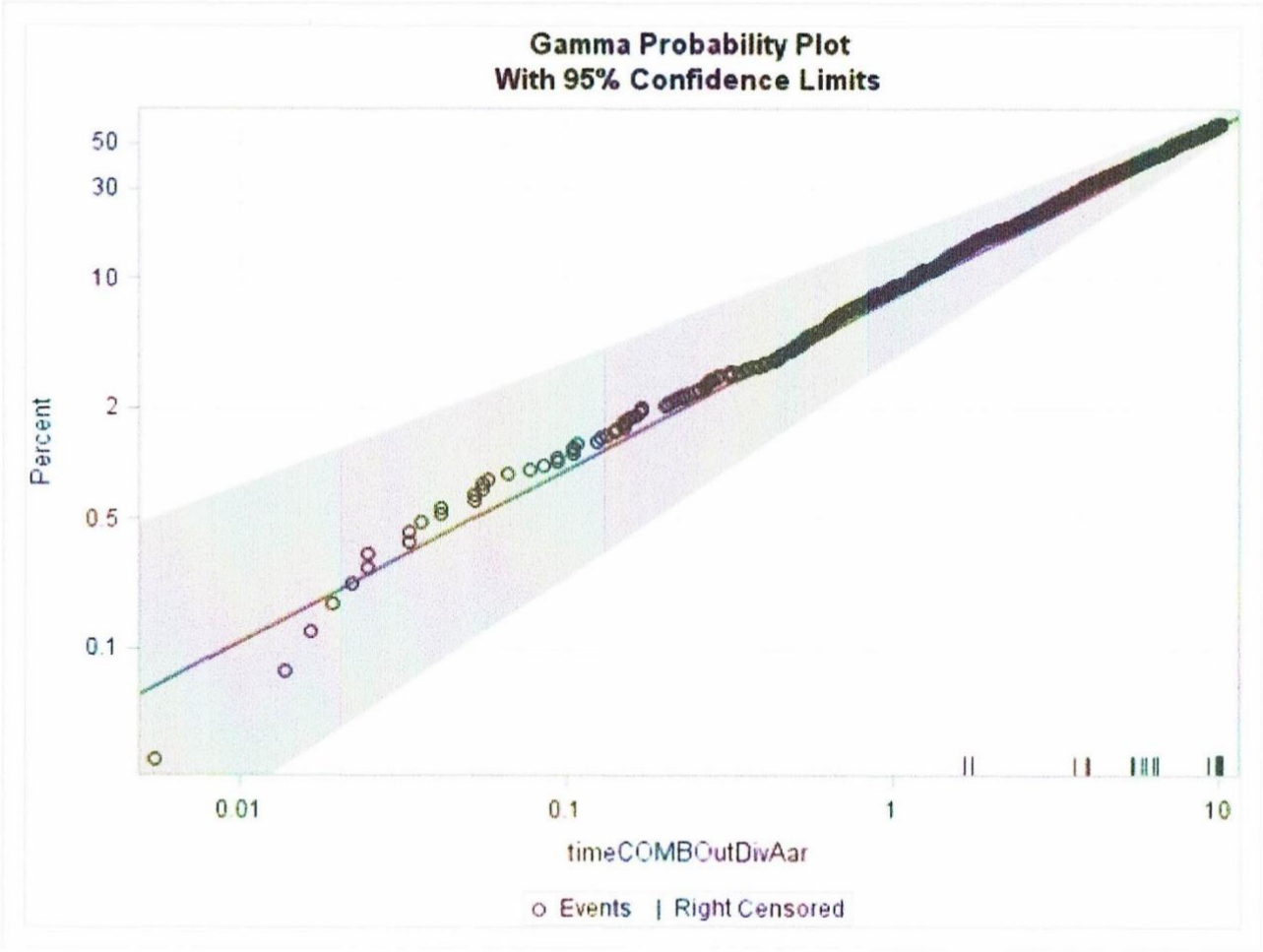

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The parametric model fitted the data reasonably well (see figure 1S A and B). The distribution of years to outcome using the accelerated failure model where the error term is modelled using the general gamma distribution showed that for both outcomes all values were within the 95% confidence limits. However, in case of all-cause death (see figure 1S A) the distribution was upwards biased but still within the 95% confidence limits.

It is noted that the results obtained with the parametric model (column 7 Tables S3 and S4) are not dramatically different from the corresponding results in column 6, when this theoretically equally valid model is used. When only the three significant predictors log(OPG/ng/L), (log (proBNP/ng/L), and log (hs-cTnT/ng/L)) were used in the Cox model in place of all 10 (column 8), the results were practically unaffected (compare columns 8 and 6).

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<Winkel P. & al. * Supplementary file S1>

Table 4S the composite outcome of AMI, UAP, CeVD, and all-cause death. Correct predictions of favorable (no outcome so far) and unfavorable status made at 3, 6 and 9 years. Cox model: four covariate scenarios as in Table 4; and parametric model (column 7) for comparison with column 6. Note that log (OPG) qualified for inclusion in column 8.

(1) Number of predictions made	(2) Time at which prediction was made	(3) Correctly predicted patient status	(4) Data without covariates included Both models N (%)	(5) Data including Standard predictors as covariates Cox model N (%)	(6) Data including Standard predictors + advanced biochemical predictors as covariates Cox model N (%)	(7) Data including Standard predictors + advanced biochemical predictors as covariates Parametric model N (%)	(8) Data including Standard predictors + Log(OPG/ng/L), Log(hs-cTnT/ng/L), and log(proBNP/ng/L) as covariates Cox model N (%)
1996	Three years	Favorable status	1514 (75.9)	1471 (73.7)	1464 (73.3)	1479 (74.1)	1463 (73.3)
		Unfavorable status	0 (0)	51 (2.56)	77 (3.86)	57 (2.86)	76 (3.81)
1989	Six years	Favorable status	1144 (57.5)	935 (47.0)	920 (46.3)	916 (46.1)	925 (46.5)
		Unfavorable status	0 (0)	349 (17.5)	370 (18.6)	368 (18.5)	367 (18.5))
1987	Nine years	Favorable status	0 (0)	504 (25.4)	542 (27.3)	550 (27.7)	549 (27.6)
		Unfavorable status	1115 (56.1)	774 (39.0)	792 (39.9)	803 (40.4)	789 (39.2)
5972	All three times combined	Favorable status	2658 (44.5)	2910 (48.7)	2926 (49.0)	2945 (49.3)	2937 (49.2)
		Unfavorable status	1115 (18.7)	1174 (19.7)	1239 (20.7)	1228 (20.6)	1222 (20.5)
		Total	3773 (63.2)	4084 (68.4)	4165 (69.7))	4173 (69.9)	4159 (69.6)

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Table 4S shows the results corresponding to Table 3S obtained when the composite outcome was used. Including the 'standard predictors' in the model increases the percent correct predictions from 63.2 (see column 4, Table 4S) to 68.4 (see column 5, Table 4S), i.e. an increase of 5.2%. Adding the 10 newer biomarkers to the model increases the number of correct predictions by 1.3%. Using the parametric model does not change the results appreciably and neither does a reduction of the biomarkers to include only the three significant ones.

Legend to figure 1S

Figure 1S A Distribution of years to death using the accelerated failure model where the error term is modelled using the general gamma distribution.

Figure 1S B Distribution of years to composite outcome (AMI, UAP, CeVD, death) using the accelerated failure model where the error term is modelled using the general gamma distribution.

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STROBE Statement items 1 to 12

Title and abstract	Item no	Recommendation	
	1	(a) Design in title	See page 1 the term 'placebo receiving' implies that controls from a trial were used
		(b) Abstract: informative and balanced summary of what was done and found	See abstract methods and results sections p 2 and p 3
Introduction			
Background/rationale	2	Scientific background and rationale	See introduction first section on page 5
Objectives	3	objectives	See introduction last section page 5
Methods			
Study design	4	Key elements of study design	See first section on the patients in material page 5
Setting	5	Setting, location, relevant dates, period of recruitment, follow-up, and data collection	See first section on the patients in material page 5 and the two sections on predictors and on the outcomes pages 6 and 7
Participants	6	(a) Cohort study eligibility, selection, follow-up	See first section on page 6 and introduction page 4 second section
Variables	7	Outcomes, predictors	See section 'the outcomes' on page 7 and the section on predictors on page 6 and 7, and table 1
Data sources/measurement	8	Sources of data, methods of assessment	See section on the outcomes on page 7 and the section on predictors on page 6 and 7, and table 1 plus references to methods.
Bias	9	Addressing potential sources of bias	See page 9 second section from above. Assessment of the potential bias due to missing values.
Study size	10	How study size was arrived at	See Hansen S et al: the CLARICOR trial design. HeartDrug 2001; 1:14-9
Quantitative variables	11	How quantitative variables were handled	They were all handled as continuous variables except for PAP-A which was dichotomized into normal vs elevated values (see table 1 and page 7 line 3)
Statistical methods	12	Statistical methods	See 'statistical analysis'

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STROBE Statement items 1 to 12

			page 8
		Missing data	See item 9
		Loss to follow-up	See page 6 last line of first section

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STROBE Statement items 13 to 22

Results			
	Item no	Recommendation	
Participants	13	Flow diagram during enrolment, randomisation, and follow-up in original trial of 2006.	See BMJ 2006;332;22-27 (paper is enclosed)
Descriptive data	14	(a) Characteristics of study participants (b) Number of participants with missing data for each variable (c) Summary of follow-up time	(a) See table 1 (b) See table 1 (c) See page 9 line 3 to5
Outcome data	15	Number of outcome events	See page 9 line 11 to 14
Main results	16	(a) Hazard rates (b) Results of predictions	(a) See tables 2, 3 (b) See tables 4 and 5
Other analyses	17	interaction	See inferential impact of the newer biomarkers page 9 first 3 lines
Discussion			
Key results	18	Summary of key results	See discussion page 11 first section
limitations	19	(a) Positive bias due to development of model and test of model using same data (b) Methodology (c) Selection bias (d) Prognosis may be worse than at present time (e) Only questionnaire data were collected at randomisation	(a) See page 12 last two lines 6 to 13 (b) See section on methodology page 12 (c) See limitations page 13 first two sections (d) See last 6 lines on page 13 and first two at page 14 (e) See page 14 line 3 to 14
Interpretations	20		See last section of discussion page 14
Generalisability	21		See (a), (b), (c), and (d) item 19
Other information			
Funding	22		See the section on acknowledgements and section on funding, both on page 19

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

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1

Prognostic value of 12 novel cardiological biomarkers in stable coronary 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 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

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S) were not associated (P ≥ 0.01) with the outcomes, not even as single predictors. Adding the remaining ten biomarkers (high-sensitive assay cardiac troponin T; neutrophil gelatinase-associated lipocalin; osteoprotegerin; N-terminal pro-B-type natriuretic peptide; tumor necrosis factor receptor 1 and 2; PAPP-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 heart disease, the addition of 10 novel biomarkers to the prediction model improved the correct prediction of all-cause death and the composite outcome by less than 1.5%.

Trial registration ClinicalTrials.gov, NCT00121550. Date of registration 13 July 2005

Date of enrolment of first participant 12 October 1999

Keywords CLARICOR, cardiovascular disease, cardiovascular risk prediction, ischaemic heart disease, predictors, mortality.

Strengths and limitations of this study

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

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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 cardiovascular 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 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

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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) sTNFR1 and (10) 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 cardiovascular diseases.

All of these have been claimed to add some prognostic information in patients with stable coronary artery heart 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 acute myocardial infarction (AMI), unstable angina pectoris (UAP), cerebrovascular vascular disease (Ce-VD) and death.

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MATERIAL

The patients

The study population is the placebo patients from the CLARICOR-study.[3,4] Patients aged 18 to 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-days 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 cardiovascular 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 acute myocardial infarction or unstable angina pectoris within the previous 3 months; (2) had had intra-coronary interventions within the previous 6 months; (3) had impaired renal function; (4) had hepatic dysfunction; (5) had congestive heart failure (New York Heart Association (NYHA) 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.

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The predictors

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

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 −80 degrees C. The quantities measured include lipoproteins,[16] high-sensitivity-C-reactive-protein/mg/L (hs-CRP/mg/L),[7] and glomerular filtration /rate/mL/min (GFR/mL/min) using creatinine.[17] Along with variables already mentioned, these quantities are those collectively referred to as 'standard predictors'.

Biomarkers included as newer biomarkers were YKL40/µg/L)[8]; high-sensitive assay cardiac troponin T/ng/L (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]; N-terminal pro-B-type natriuretic peptide/ng/L (proBNP/ng/L)[9]; cathepsin-B/µg/L[6,18]; endostatin/ng/mL[19]; cathepsin-S/µg/L[6,20]; soluble TNF receptor 1/pg/mL and soluble TNF receptor 2/pg/mL (sTNFR1/pg./mL and sTNFR2/pg/mL)[5,21]; neutrophil gelatinase-associated lipocalin/ng/L (NGAL/ng/L)[22]; calprotectin/mg/L[11]; and osteoprotegerin/ng/L (OPG/ng/L)[12]. Due to storage problems some marker data are missing on some patients.

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

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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 December 31, 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: acute myocardial infarction (AMI), unstable angina pectoris (UAP), cerebrovascular disease (CeVD), or all-cause death.

METHODS

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 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].

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We also analysed data using a parametric, accelerated failure-time model using the generalized 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 hazard ratio is the factor by which the hazard increases when the logarithm increases by 1, i.e., when the concentration increases by a factor e = 2.72.

Biomarkers with a hazard ratio with P value $\geq$ 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 percent 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]

Secondly, we report the areas under the ROCs (receiver operating characteristics), also known as 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

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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.

Ethics and safety

Ethics approval and consent to participate was given by VEKKF01-076/99; Danish Medicines Agency 2612-975; Danish Data Protection Agency 1999-1200-174; VEK H-B-2009-015.

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. The public involvement was trough the approvals given by the regional ethics committee (KF 01-076/99 and journal no. H-12012125), the Danish Medicines Agency (2612-975), and the Danish Data Protection Agency (1999-1200-174).

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RESULTS

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. Abbreviations as in section on abbreviations.

Quantity	Distribution
Demographics and previous history	
Sex (male) N (%)	1518 (69.0%)
Age/year mean (SD)	65.2 (10.4) 2199
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[a]	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 (Tri-glyceride/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 (proBNP/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 ≥ 4mIU/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

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FOOTNOTES

a) The value of N varies because the laboratory tests have missing values (mostly due to storage problems). log: natural logarithm.

Table 1 presents an overview of the covariates expected to be available from stable cardiovascular disease patients 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. The composition of the two groups appears consistent.

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 supplementary file S1, 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) in the subsequent regression equation (see table 2S in supplementary file S1). The latter equation now included the 'standard predictors', plus the remaining 10 newer biomarkers and the above-mentioned time dependent covariates. It appears from

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supplementary table 2S that only log (proBNP/ng/L), log (hs-cTnT/ng/L), and log (OPG/ng/L) contributed significantly to the prediction.

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

Model and covariates included in model	**Total number of predictions made per outcome**	**Number and percent of correct predictions of events**	
		All-cause death N (%)	**Composite of AMI[a] ,UAP[b], Ce-VD[c], and 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)

a) AMI acute myocardial infarction
b) UAP unstable angina pectoris
c) Ce-VD cerebrovascular disease

Table 2 (see also supplementary file S1 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 percent 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

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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.

Using the parametric model in place of the Cox model we obtained quite similar results (see tables 3S and 4S in supplementary file S1 and figure 1S A-B). The same was true if we only included log (proBNP/ng/L), log (hs-cTnT/ng/L), and log (OPG/ng/L) instead of all 10 biomarkers when the Cox model was used (see tables 3S and 4S in the supplementary file S1).

Table 3. *C*-indices. Cox model estimates applied to the 0-9-year follow-up window (*n* = 1998).

	Binary-outcome *C* (AUC), observed (predicted)[a]	**Dynamic *C*,** Observed[b]
Composite outcome[c] (1115 events)		
Standard predictors (SP) only	0.711 (0.707)	0.640
The 10 newer markers & SP	0.732 (0.732)	0.657
Log(hsTnT/ng/L) + log(proBNP/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 & SP	0.824 (0.816)	0.765
Log(hsTnT/ng/L) + log(proBNP/ng/L) + SP	0.821 (0.813)	0.762

a) The 'observed' AUCs summarize 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.
b) Analogous concordance rate between time to event and predicted risk.
c) Composite outcome: first occurrence of acute myocardial infarction. unstable angina pectoris, cerebrovascular disease or death. SP 'standard predictors', see table1.

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Table 3 summarizes 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(hsTNT/ng/L), and log(proBNP/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.

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 heart disease, 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 less than 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, enrollment took place 10 years later than in the CLARICOR trial and the incidence of cardiovascular 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 to only 41% in the CLARICOR material). So, the age of our material is a weakness.

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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 to 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 coronary heart disease are likely going to be worse than observed here.

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 would a sophisticated integral over continuous time.

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).

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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 coronary heart disease 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 stable coronary heart disease patients as here defined include the fact that only questionnaire data

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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 cardiovascular risk assessment in asymptomatic people not suffering from CAD.[35]

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 proBNP, possibly supplemented by osteoprotegerin.

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Abbreviations

AMI: acute myocardial infarct; Apo A1: apoprotein A1; Apo B: apoprotein B; CeVD: cerebro-vascular disease; Chol-HDL: cholesterol high density lipoprotein; Chol-LDL: cholesterol low density lipoprotein; CLARICOR: Clarithromycin for patients with stable coronary heart disease; CRP: c-reactive protein; GFR: Glomerular filtration rate; hs-cTnT: High-sensitive assay cardiac troponin T; NGAL: neutrophil gelatinase-associated lipocalin; NYHA: New York Heart Association; OPG: osteoprotegerin; PREMAC: Predictors for major cardiovascular outcomes in stable ischaemic heart disease; proBNP: N-terminal pro-B-type natriuretic peptide;

TNFR1: tumor necrosis factor receptor 1; TNFR2: tumor necrosis factor receptor 2; UAP: unstable angina pectoris.

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Availability of data and materials

All pertinent anonymised data will be uploaded at ZENODO (http://zenodo.org/) when the individual manuscripts have been published.

Authors' contributions

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, 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

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resolved. PW, JCJ, JH, GBJ, EK, AS, JK, HJK, KKI, MB, AL, JÄ, CG contributed substantially to the interpretation of the data.

Competing interests

The authors declare that they have no competing interests.

REFERENCES

1. Winkel P, Jakobsen JC, Hilden J, et al. Prognostic value of routinely available data in patients with stable coronary heart disease. A 10-year follow-up of patients sampled at random times during their disease course. Open Heart 2018:e000808. Doi:10.1136/openhrt-2018-000808

2. Winkel P, Jakobsen JC, Hilden J, Lange T, Jensen GB, Kjøller E, et al: Predictors for major cardiovascular outcomes in stable ischaemic heart disease (PREMAC): statistical analysis plan for data originating from the CLARICOR (clarithromycin for patients with stable coronary heart disease) trial: Diagnostic and Prognostic Research (2017) 1:10 DOI 10.1186/s41512-017-0009-y.

3. Jespersen CM, Als-Nielsen B, Damgaard M, Hansen JF, Hansen S, Helø OH, et al: Randomised placebo controlled multicentre trial to assess short term clarithromycin for patients with stable coronary heart disease: CLARICOR trial. BMJ. 2006; 332:22–7.

4. Hansen S, Als-Nielsen B, Damgaard M, Helø OH, Petersen L, Jespersen CM, et al: Intervention with clarithromycin in patients with stable coronary heart disease: the CLARICOR trial design. Heart Drug. 2001; 1:14–9.

5. Carlsson AC, Ruge T, Kjøller E, Hilden J, Kolmos HJ, Sajadieh A o. a. 10-year associations between tumor necrosis factor receptors 1 and 2 and cardiovascular events in patients with stable coronary heart disease: a CLARICOR (effect of clarithromycin on

For peer review only

mortality and morbidity in patients with coronary heart disease) trial substudy. Journal of the American Heart Association 2018 apr 23;7(9): e 008299 Doi:10. 116/JAHA 127.008299

6. Wuopio J, Hilden J, Bring C, Kastrup J, Sajadieh A, Jensen GB o.a. Cathepsin B and S as markers for cardiovascular risk and all-cause mortality in patients with stable coronary heart disease during 10 years: a CLARICOR trial substudy. Atherosclerosis. 2018 nov 1:278: 97 to 102. Tilgængelig fra, DOI: 10. 1016/j atherosclerosis. 2018.09.006

7. Harutyunyan MJ, Mathiasen AB, Winkel P, Gøtze JP, Hansen JF, Hildebrandt P, et al: High-sensitivity C-reactive protein and N-terminal pro-B-type natriuretic peptide in patients with stable coronary heart disease: a prognostic study within the CLARICOR trial. Scand J Clin Lab Invest. 2011; 71:52–62.

8. Schroder J, Jakobsen CJ, Winkel P, Hilden J, Jensen GB, Sajadieh A, et al. Prognosis and Reclassification by YKL-40 in Stable Coronary Artery Disease. J Am Heart Assoc. 2020;9: e014634. DOI: 10.1161/JAHA.119.014634.

9. Lyngbaek S, Winkel P, Gotze JP, Kastrup J, Gluud C, Kolmos HJ, et al. Risk stratification in stable coronary artery disease is possible at cardiac troponin levels below conventional detection and is improved by use of N-terminal pro-B-type natriuretic peptide. European Journal of Preventive Cardiology 2014; 21:1275-1284.

10. Iversen KK, Teisner B, Winkel P, Gluud C, Kjoller E, Kolmos HJ, et al. Pregnancy associated plasma protein-A as a marker for myocardial infarction and death in patients with stable coronary artery disease: a prognostic study within the CLARICOR Trial. Atherosclerosis 2011; 214:203-208.

For peer review only

11. Larsen SB, Grove EL, Pareek M, Kristensen SD, Hvas AM. Calprotectin and platelet aggregation in patients with stable coronary artery disease. PloS ONE 2015; 10(5):e0125992.

12. Bjerre M, Hilden J, Kastrup J, Skoog M, Hansen JF, Kolmos HJ, et al. Osteoprotegerin independently predicts mortality in patients with stable coronary artery disease: the CLARICOR trial. Scandinavian Journal of Clinical and Laboratory Investigation 2014; 74:657-664.

13. Winkel P, Hilden J, Hansen JF, Kastrup J, Kolmos HJ, Kjoller E, et al. Clarithromycin for stable coronary heart disease increases all-cause and cardiovascular mortality and cerebrovascular morbidity over 10years in the CLARICOR randomised, blinded clinical trial. International Journal of Cardiology 2015, 182:459-465.

14. Winkel P, Hilden J, Fischer Hansen J, Hildebrandt P, Kastrup J, Kolmos HJ, et al: Excess sudden cardiac deaths after short-term clarithromycin administration in the CLARICOR trial: why is this so, and why are statins protective? Cardiology. 2011; 118:63–67.

15. Gluud C, Als-Nielsen B, Damgaard M, Fischer Hansen J, Hansen S, Helø OH, et al: Clarithromycin for 2 weeks for stable coronary heart disease: 6-year follow-up of the CLARICOR randomized trial and updated meta-analysis of antibiotics for coronary heart disease. Cardiology. 2008; 111:280

For peer review only

25

16. Helmersson-Karlqvist J, Ridefelt P, Lind L, Larsson A. Reference values for 34 frequently used laboratory tests in 80-year-old men and women. Maturitas. 2016; 92: 97-101.

17. Inker LA, Schmid CH, Tighiouart H, Eckfeldt JH, Feldman HI, Greene T, et al: Estimating glomerular filtration rate from serum creatinine and cystatin C. N Engl J Med. 2012;367:20–9.

18. Wang N, Bai X, Jin B, Han W, Sun X, Chen X. The association of serum cathepsin-B concentration with age-related cardiovascular-renal subclinical state in a healthy Chinese population. Arch Gerontol Geriatr. 2016; 65:146-55.

19. Arnlov J, Ruge T, Ingelsson E, Sundstrom J, Lind L. Serum endostatin and risk of mortality in the elderly: findings from 2 community-based cohorts. Arterioscler Thromb Vasc Biol.2013, 33: 2689-95.

20. Jobs E, Ingelsson E, Risérus U, Nerpin E, Jobs M, Sundström J, et al. Association between serum cathepsin S and mortality in older adults. JAMA. 2011; 306:1113-21.

21. Carlsson AC, Larsson TE, Helmersson-Karlqvist J, Larsson A, Lind L, Ärnlöv J. Soluble TNF receptors and kidney dysfunction in the elderly. J Am Soc Nephrol. 2014;25:1313-20.

22. Helmersson-Karlqvist J, Larsson A, Carlsson AC, Venge P, Sundstrom J, Ingelsson E, et al. Urinary neutrophil gelatinase-associated lipocalin (NGAL) is associated with mortality in a community-based cohort of older Swedish men. Atherosclerosis 2013; 227:408-13.

For peer review only

23. Kjøller E, Hilden J, Winkel P, Frandsen NJ, Galatius S, Jensen G, et al: Good interobserver agreement was attainable on outcome adjudication in patients with stable coronary heart disease. J Clin Epidemiol. 2012; 65:444–53.

24. Kjøller E, Hilden J, Winkel P, Galatius S, Frandsen NJ, Jensen GB, et al: Agreement between public register and adjudication committee outcome in a cardiovascular randomized clinical trial. Am Heart J. 2014; 168:197–204.ok

25. Helweg-Larsen K: The Danish register of causes of death. Scand J Public Health. 2011;39(7 30. Suppl):26-9.

26. Lynge E, Sandegaard JL, Rebolj M: The Danish National Patient Register. Scand J Public Health. 2011;39(7 suppl):30–3.

27. Lin DY, Wei LJ, Ying Z: Checking the Cox model with cumulative sums of martingale-based residuals. Biometrika. 1993; 80:557–72.

28. Allison PD: Survival Analysis Using SAS: A Practical Guide, second edition 2010 SAS institute Inc. Cary, NC, USA.

29. Harrell FE Jr, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. Stat Med. 1996;15: 361-87.

For peer review only

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30. Pencina MJ, d'Agostino RB. Overall C as a measure of discrimination in survival analysis: model specific population value and confidence interval estimation. Stat Med. 2004; 23: 2109 -23.

31. Little R: A test of missing completely at random for multivariate data with missing values. J Am Stat Assoc. 1988; 83:1198–1202.

32. Steg PG, Greenlaw N, Tendera M, et al. Prevalence of anginal symptoms and myocardial ischemia and their effect on clinical outcomes in outpatients with stable coronary artery disease. Data from the International Observational CLARIFY Registry. JAMA Intern Med. 2014;174(10):1651-1659. doi:10.1001/jamainternmed.2014.3773

33. Schmidt M, Jacobsen JB, Lash TL, Bøtker HE, Sørensen HT: 25-year trends in first time hospitalisation for acute myocardial infarction, subsequent short and long-term mortality, and the prognostic impact of sex and comorbidity: a Danish nationwide cohort study. BMJ. 2012;344: e356.

34. Solomon SD, Claggett B,Desai AS, *et al*. Influence of ejection fraction on outcomes and efficacy of sacubitril/valsartan (lcz696) in heart failure with reduced ejection fraction. Circulation 2016;9:e00274.

35. Scott IA. Evaluating cardiovascular risk assessment in asymptomatic people.BMJ 2009:a2844doi:10.1136/bmj.a2844

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Supplementary file S1

Table 1S All-cause mortality hazard ratios (HR) of newer biochemical predictors not routinely used in clinical work when each of these predictors is used alone (columns 2 to 4), and when it is used in combination with the 'standard predictors'[a] (column 5 to 7). Two of them were then discarded and each of the remaining 10 was assessed when used in combination with the standard predictors and the remaining 9 of the 10 newer biochemical predictors selected among the 12 candidates (columns 8 to 10).

Newer biochemical candidate predictor	When candidate predictor is the only predictor included in the model (stratified by centre)			When 'standard predictors' is added to the model (stratified by centre)			When in addition the 10 selected predictors are added to the model (stratified by centre)		
	HR[b]	95% CI	P	HR	95% CI	P	HR	95% CI	P
log (endostatin/ng/mL)	3.49	2.81 to 4.33	<0.0001	1.75	1.34 to 2.27	<0.0001	1.23	0.92 to 1.63	0.16
log (OPG/ng/L)	3.37	2.88 to 3.94	<0.0001	1.68	1.35 to 2.09	<0.0001	1.21	0.97 to 1.63	0.092
log (sTNFR1/pg/mL)	3.80	3.19 to 4.54	<0.0001	1.84	1.46 to 2.33	<0.0001	1.10	0.81 to 1.48	0.55
og (sTNFR2/pg/mL)	5.45	4.40 to 6.76	<0.0001	2.39	1.80 to 3.18	<0.0001	1.43	0.99 to 2.07	0.056
log(proBNP/ng/L)	1.76	1.66 to 1.87	<0.0001	1.44	1.34 to 1.55	<0.0001	1.28	1.19 to 1.39	<0.0001
log(hs-cTnT/ng/L)	2.31	2.16 to 2.47	<0.0001	1.73	1.56 to 1.92	<0.0001	1.46	1.30 to 1.65	<0.0001
PAPP-A_binary[c]	1.84	1.53 to 2.21	<0.0001	1.39	1.15 to 1.68	0.0007	0.85	0.69 to 1.03	0.10
log (YKL40/µg/L)	1.76	1.59 to 1.95	<0.0001	1.32	1.17 to 1.49	<0.0001	1.10	0.97 to 1.25	0.15
log (NGAL/ng/L)	1.33	1.12 to 1.57	0.0011	1.03	0.85 to 1.24	0.78	0.90	0.74 to 1.10	0.30
log(Calprotectin/)	1.08	0.95 to 1.23	0.25	1.02	0.89 to 1.18	0.74	Not included in analysis		
log (Cathepsin-B/µg/L)	2.81	2.40 to 3.28	<0.0001	1.43	1.19 to 1.73	0.0002	1.09	0.89 to 1.33	0.42
log (Cathepsin-S/µg/L)	1.12	0.86 to 1.47	0.40	1.10	0.83 to 1.45	0.51	Not included in analysis		

a) The standard predictors are shown in Table 1.

b) Hazard ratio associated with unit increase on log scale, except for PAPP-A (binary).

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c) Binary quantity. 1: PAPP-A was ≥ 4 mIU/L, 0: PAPP-A was < 4 mIU/L.

1. Inferential impact of the newer biomarkers

As the proportional hazard's assumption was violated for age[29] and age interacted significantly with time since randomisation, we included an interaction between age at entry and time (since randomisation) in the inference analyses.

Table 1S shows the results of a Cox regression of all-cause death on each of the 12 biomarkers when the biomarker was used alone as a covariate (columns 2 through 4), and when it was used in combination with the 'standard predictors' (columns 5 through 7).

Columns 8 through 10 in Table 1S shows the result of a regression of the outcome on the 'standard predictors' and the 10 best biochemical predictors. Now only log (proBNP /ng/L) and log(hs-cTnT/ng/L) have a HR significantly (P < 0.01) different from 1. Log(calprotectin/mg/L) and log(cathepsin-S/µg/L) did not have an inferential impact (P < 0.01 not attained), not even when used alone.

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Table 2S the composite outcome (comprising first occurrence of acute myocardial infarction, unstable angina pectoris, cerebro-vascular disease, and death). Hazard ratios of each of 13 biochemical predictors not routinely used in clinical work when each of these predictors is used alone (columns 2 to 4), and when it is used in combination with the 'standard predictors' (columns 5 to 7). Two of them were then discarded and each of the remaining 11 was assessed when used in combination with the standard predictors and the remaining 10 of the 11 newer biochemical predictors selected among the 13 candidates (columns 8 to 10)

Newer biochemical candidate predictor	When candidate predictor is the only predictor included in the model (stratified by centre)			When 'standard predictors' is added to the model (stratified by centre)			When in addition the 11[a] selected predictors are added to the model (stratified by centre)		
	HR	95% CI of HR	P	HR	95% CI of HR	P	HR	95% CI of HR	P
log (Endostatin/ng/mL)	2.18	1.84 to 2.58	<0.0001	1.44	1.17 to 1.72	0.0006	1.23	0.99 to 1.54	0.062
log (OPG/ng/L)	1.34	1.05 to 1.71	0.019	0.94	0.70 to 1.26	0.67	0.78	0.58 to 1.04	0.094
log (OPG/ng/L) ·time/year[b]	1.11	1.06 to 1.16	<0.0001	1.09	1.03 to 1.16	0.0022	1.104	1.044 to 1.168	0.0005
log (sTNFR1/pg/mL)	2.14	1.86 to 2.46	<0.0001	1.33	1.11 to 1.60	0.0021	1.05	0.84 to 1.32	0.67
log (sTNFR2/pg/mL)	2.56	2.15 to 3.03	<0.0001	1.49	1.19 to 1.85	0.0004	1.13	0.85 to 1.50	0.40
log (proBNP/ng/L)	1.37	1.31 to 1.44	<0.0001	1.26	1.19 to 1.33	<0.0001	1.18	1.11 to 1.25	<0.0001
log (hs-cTnT/ng/L)	1.83	1.70 to 1.97	<0.0001	1.49	1.35 to 1.64	<0.0001	1.31	1.17 to 1.46	<0.0001
PaPP-A (binary)[c]	1.45	1.24 to 1.70	<0.0001	1.24	1.06 to 1.46	0.0077	0.89	0.75 to 1.05	0.15
log (YKL40/µg/L)	1.35	1.24 to 1.47	<0.0001	1.13	1.03 to 1.24	0.013	1.01	0.91 to 1.11	0.93
log (NGAL/ng/L)	1.23	1.08 to 1.40	0.0023	1.03	0.89 to 1.19	0.73	0.97	0.84 to 1.13	0.74
log (Calprotectin/)	1.06	0.95 to 1.17	0.32	1.00	0.90 to 1.12	0.95	Not included in analysis		
log (cathepsin-B/µg/L)	1.70	1.50 to 1.93	<0.0001	1.17	1.01 to 1.35	0.040	0.99	0.85 to 1.16	0.92
log (cathepsin-S/µg/L)	1.06	0.86 to 1.31	0.59	0.98	0.79 to 1.22	0.88	Not included in analysis		

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a) Note that now a time dependent covariate has been added [log (OPG/ng/L) · time/year] to the 10 original predictors.

b) log (OPG/ng/L) significantly violated the proportional hazard assumption. We found a significant linear relationship between log (OPG/ng/L) and time since randomisation which may explain the violation. The product of log (OPG/ng/L) and time/year was therefore included in the inference analysis. However, when the Cox model is used for prediction, time dependent covariates are not allowed (SAS 9.4). Therefore, in the latter context we only include log (OPG/ng/L).

c) Binary quantity. 1: PAPP-A was ≥4 mIU/L, 0: PAPP-A was <4 mIU/L.

Table 2S corresponds to Table 1S except that the outcome is the composite outcome. It is noted that a time-dependent covariate is now included because log (OPG/ng/L) violated the proportional hazard assumption. This was remedied by including the covariate log (OPG/ng/L) ·time/year. It is seen that when all the biomarkers were included in the Cox analysis log(OPG/ng/L)·time/year, log(proBNP/ng/L), and log(hs-cTnT/ng/L) were the only ones which had a P value below the threshold of 0.01. Again log(calprotectin/mg/L) and log(cathepsin-S/µg/L) could be excluded from the final analysis, the result of which is shown in columns 8 through 10.

2. Practical impact of the novel biomarkers

Table 3S All-cause death. Correct predictions of favorable (alive) and unfavorable (not alive) status made at 3 years, at 6 years, and at 9 years following randomisation in the 1998 placebo patients from the CLARICOR trial. Four covariate scenarios were examined with Cox regression (see text of columns 4, 5, 6, and 8). For comparison with the results of column 6, column 7 shows the corresponding results when the accelerated failures model was used.

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(1) Number of predictions made	(2) Time at which prediction was made	(3) Correctly predicted patient status	(4) Data without covariates included Both models N (%)	(5) Data including Standard predictors as covariates Cox model N (%)	(6) Data including Standard predictors + advanced biochemical predictors as covariates Cox model N (%)	(7) Data including Standard predictors + advanced biochemical predictors as covariates Parametric model N (%)	(8) Data including Standard predictors log(OPG/ng/L) log (hs-cTnT/ng/L) and log(proBNP/ng/L) as covariates Cox Model N (%)
1996	Three years	Favorable status	1825 (91.4)	1821 (91.2)	1816 (91.0)	1814 (90.9)	1816 (91.0)
		Unfavorable status	0 (0.00)	10 (0.50)	19 (0.95)	14 (0.70)	19 (0.95)
1989	Six years	Favorable status	1601 (80.5)	1555 (78.2)	1551 (78.0)	1538 (77.3)	1553 (78.1)
		Unfavorable status	0 (0.00)	85 (4.27)	120 (6.03)	118 (5.93)	113 (5.68)
1987	Nine years	Favorable status	1342 (67.5)	1192 (60.0)	1219 (61.3)	1217 (61.2)	1212 (61.0)
		Unfavorable status	0 (0.00)	297 (14.9)	331(16.7)	323 (16.3)	339 (17.1)
5972	All three times combined	Favorable status	4768 (79.8)	4585 (76.8)	4586 (76.8)	4569 (76.5)	4581 (76.7)
		Unfavorable status	0 (0.00)	392 (6.56)	470 (7.87)	364 (6.10)	471 (7.89)
		Total	4768 (79.8)	4977 (83.3)	5056 (84.7)	4933 (82.6)	5052 (84.6)

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The results of the predictions of survival status made at 3 years, at 6 years, and at 9 years following randomisation in the 1998 placebo patients are summarized in Table 3S.

When the 'standard predictors' were included as covariates (column 5) for all-cause mortality, 83.3% of the predictions were correct. Adding the 10 newer biochemical predictors (column 6) the percentage was increased by 1.4% to 84.7%.

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Figure 1S A

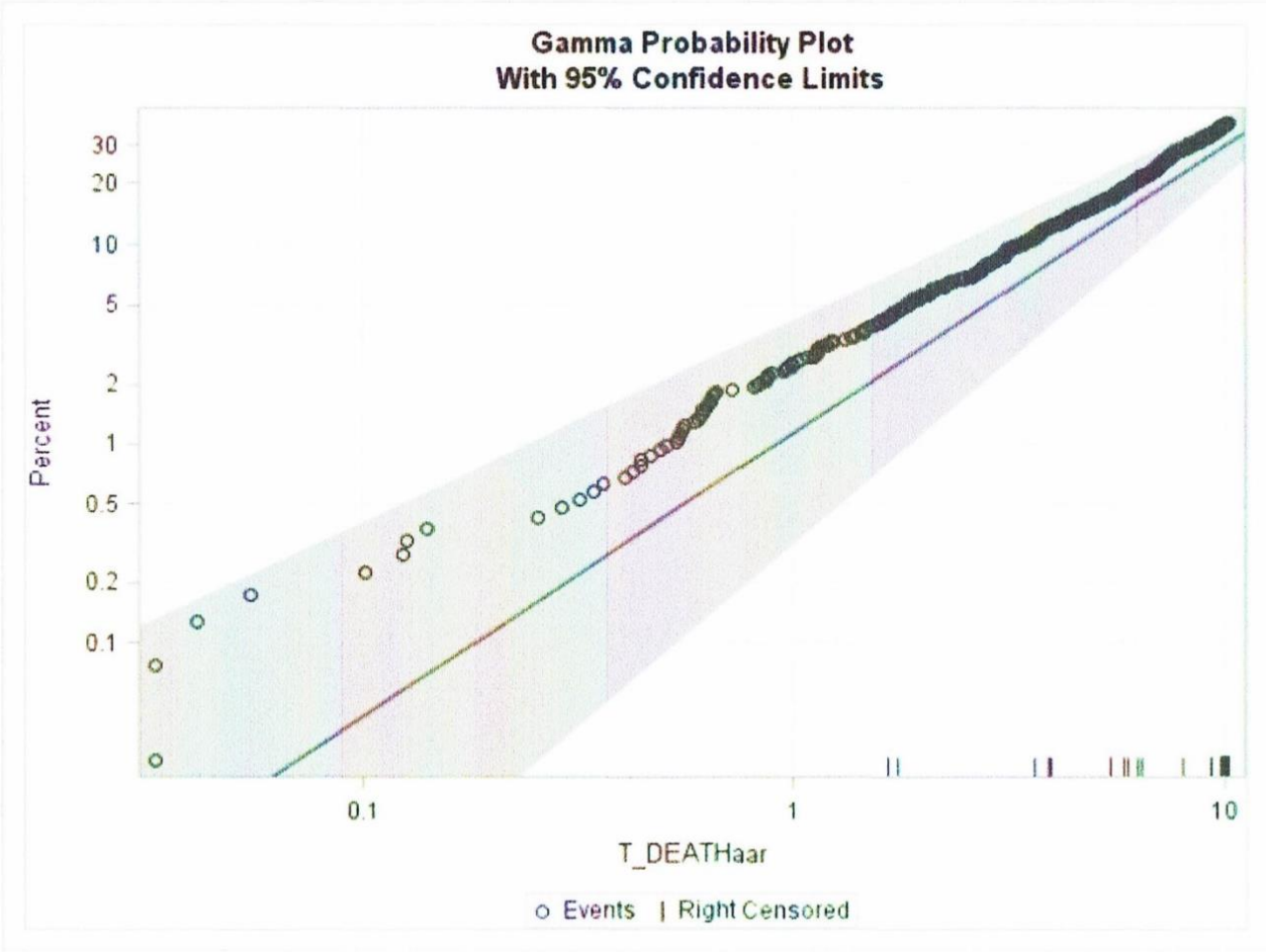

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Figure 1S B

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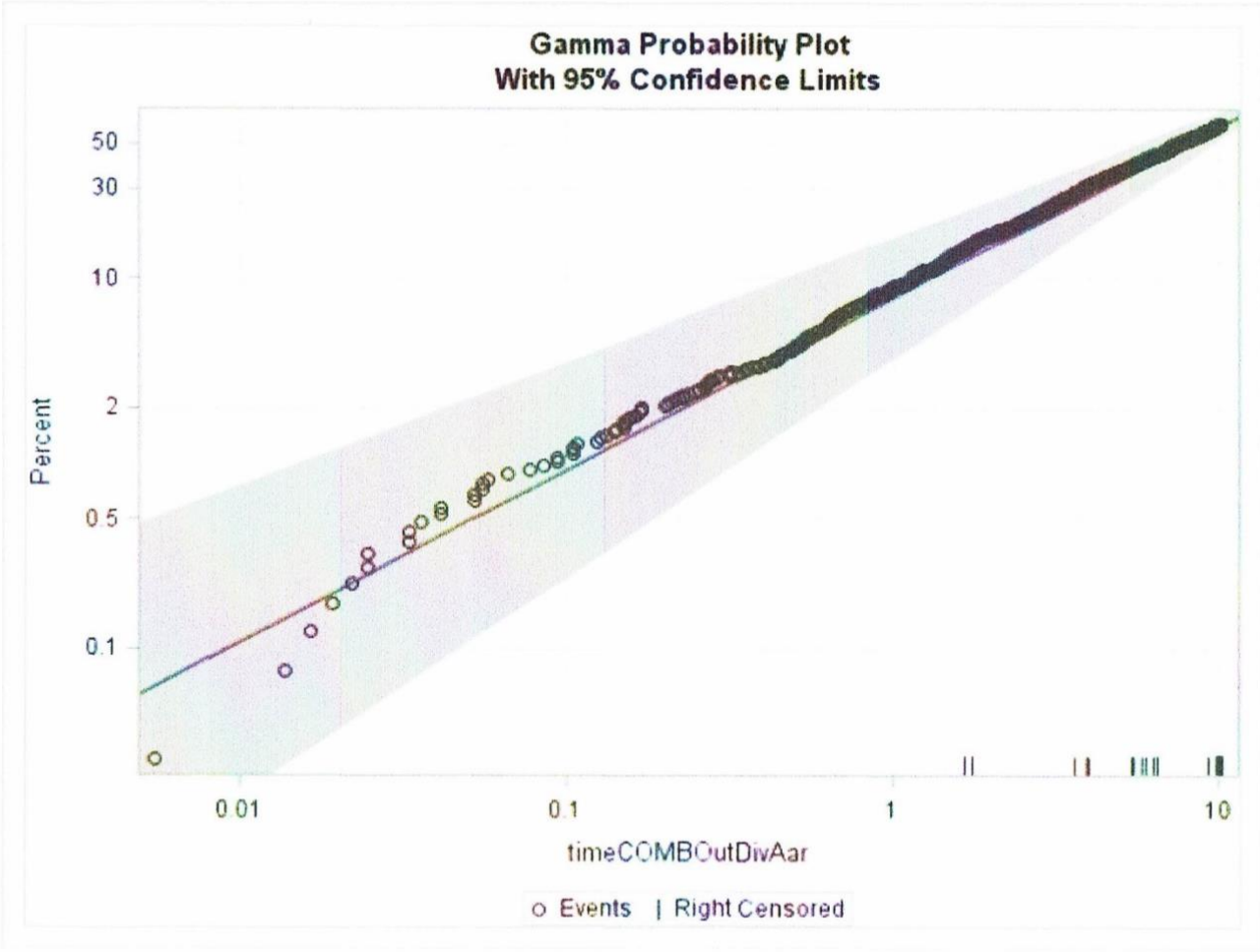

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The parametric model fitted the data reasonably well (see figure 1S A and B). The distribution of years to outcome using the accelerated failure model where the error term is modelled using the general gamma distribution showed that for both outcomes all values were within the 95% confidence limits. However, in case of all-cause death (see figure 1S A) the distribution was upwards biased but still within the 95% confidence limits.

It is noted that the results obtained with the parametric model (column 7 Tables S3 and S4) are not dramatically different from the corresponding results in column 6, when this theoretically equally valid model is used. When only the three significant predictors log(OPG/ng/L), (log (proBNP/ng/L), and log (hs-cTnT/ng/L)) were used in the Cox model in place of all 10 (column 8), the results were practically unaffected (compare columns 8 and 6).

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Table 4S the composite outcome of AMI, UAP, CeVD, and all-cause death. Correct predictions of favorable (no outcome so far) and unfavorable status made at 3, 6 and 9 years. Cox model: four covariate scenarios as in Table 4; and parametric model (column 7) for comparison with column 6. Note that log (OPG) qualified for inclusion in column 8.

(1) Number of predictions made	(2) Time at which prediction was made	(3) Correctly predicted patient status	(4) Data without covariates included Both models N (%)	(5) Data including Standard predictors as covariates Cox model N (%)	(6) Data including Standard predictors + advanced biochemical predictors as covariates Cox model N (%)	(7) Data including Standard predictors + advanced biochemical predictors as covariates Parametric model N (%)	(8) Data including Standard predictors + Log(OPG/ng/L), Log(hs-cTnT/ng/L), and log(proBNP/ng/L) as covariates Cox model N (%)
1996	Three years	Favorable status	1514 (75.9)	1471 (73.7)	1464 (73.3)	1479 (74.1)	1463 (73.3)
		Unfavorable status	0 (0)	51 (2.56)	77 (3.86)	57 (2.86)	76 (3.81)
1989	Six years	Favorable status	1144 (57.5)	935 (47.0)	920 (46.3)	916 (46.1)	925 (46.5)
		Unfavorable status	0 (0)	349 (17.5)	370 (18.6)	368 (18.5)	367 (18.5))
1987	Nine years	Favorable status	0 (0)	504 (25.4)	542 (27.3)	550 (27.7)	549 (27.6)
		Unfavorable status	1115 (56.1)	774 (39.0)	792 (39.9)	803 (40.4)	789 (39.2)
5972	All three times combined	Favorable status	2658 (44.5)	2910 (48.7)	2926 (49.0)	2945 (49.3)	2937 (49.2)
		Unfavorable status	1115 (18.7)	1174 (19.7)	1239 (20.7)	1228 (20.6)	1222 (20.5)
		Total	3773 (63.2)	4084 (68.4)	4165 (69.7))	4173 (69.9)	4159 (69.6)

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Table 4S shows the results corresponding to Table 3S obtained when the composite outcome was used. Including the 'standard predictors' in the model increases the percent correct predictions from 63.2 (see column 4, Table 4S) to 68.4 (see column 5, Table 4S), i.e. an increase of 5.2%. Adding the 10 newer biomarkers to the model increases the number of correct predictions by 1.3%. Using the parametric model does not change the results appreciably and neither does a reduction of the biomarkers to include only the three significant ones.

Legend to figure 1S

Figure 1S A Distribution of years to death using the accelerated failure model where the error term is modelled using the general gamma distribution.

Figure 1S B Distribution of years to composite outcome (AMI, UAP, CeVD, death) using the accelerated failure model where the error term is modelled using the general gamma distribution.

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STROBE Statement items 1 to 12

Title and abstract	Item no	Recommendation	
	1	(a) Design in title	See page 1 the term 'placebo receiving' implies that controls from a trial were used
		(b) Abstract: informative and balanced summary of what was done and found	See abstract methods and results sections p 2 and p 3
Introduction			
Background/rationale	2	Scientific background and rationale	See introduction first section on page 5
Objectives	3	objectives	See introduction last section page 5
Methods			
Study design	4	Key elements of study design	See first section on the patients in material page 5
Setting	5	Setting, location, relevant dates, period of recruitment, follow-up, and data collection	See first section on the patients in material page 5 and the two sections on predictors and on the outcomes pages 6 and 7
Participants	6	(a) Cohort study eligibility, selection, follow-up	See first section on page 6 and introduction page 4 second section
Variables	7	Outcomes, predictors	See section 'the outcomes' on page 7 and the section on predictors on page 6 and 7, and table 1
Data sources/measurement	8	Sources of data, methods of assessment	See section on the outcomes on page 7 and the section on predictors on page 6 and 7, and table 1 plus references to methods.
Bias	9	Addressing potential sources of bias	See page 9 second section from above. Assessment of the potential bias due to missing values.
Study size	10	How study size was arrived at	See Hansen S et al: the CLARICOR trial design. HeartDrug 2001; 1:14-9
Quantitative variables	11	How quantitative variables were handled	They were all handled as continuous variables except for PAP-A which was dichotomized into normal vs elevated values (see table 1 and page 7 line 3)
Statistical methods	12	Statistical methods	See 'statistical analysis'

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STROBE Statement items 1 to 12

			page 8
		Missing data	See item 9
		Loss to follow-up	See page 6 last line of first section

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STROBE Statement items 13 to 22

Results			
	Item no	Recommendation	
Participants	13	Flow diagram during enrolment, randomisation, and follow-up in original trial of 2006.	See BMJ 2006;332;22-27 (paper is enclosed)
Descriptive data	14	(a) Characteristics of study participants (b) Number of participants with missing data for each variable (c) Summary of follow-up time	(a) See table 1 (b) See table 1 (c) See page 9 line 3 to5
Outcome data	15	Number of outcome events	See page 9 line 11 to 14
Main results	16	(a) Hazard rates (b) Results of predictions	(a) See tables 2, 3 (b) See tables 4 and 5
Other analyses	17	interaction	See inferential impact of the newer biomarkers page 9 first 3 lines
Discussion			
Key results	18	Summary of key results	See discussion page 11 first section
limitations	19	(a) Positive bias due to development of model and test of model using same data (b) Methodology (c) Selection bias (d) Prognosis may be worse than at present time (e) Only questionnaire data were collected at randomisation	(a) See page 12 last two lines 6 to 13 (b) See section on methodology page 12 (c) See limitations page 13 first two sections (d) See last 6 lines on page 13 and first two at page 14 (e) See page 14 line 3 to 14
Interpretations	20		See last section of discussion page 14
Generalisability	21		See (a), (b), (c), and (d) item 19
Other information			
Funding	22		See the section on acknowledgements and section on funding, both on page 19

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