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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).

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' (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)

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

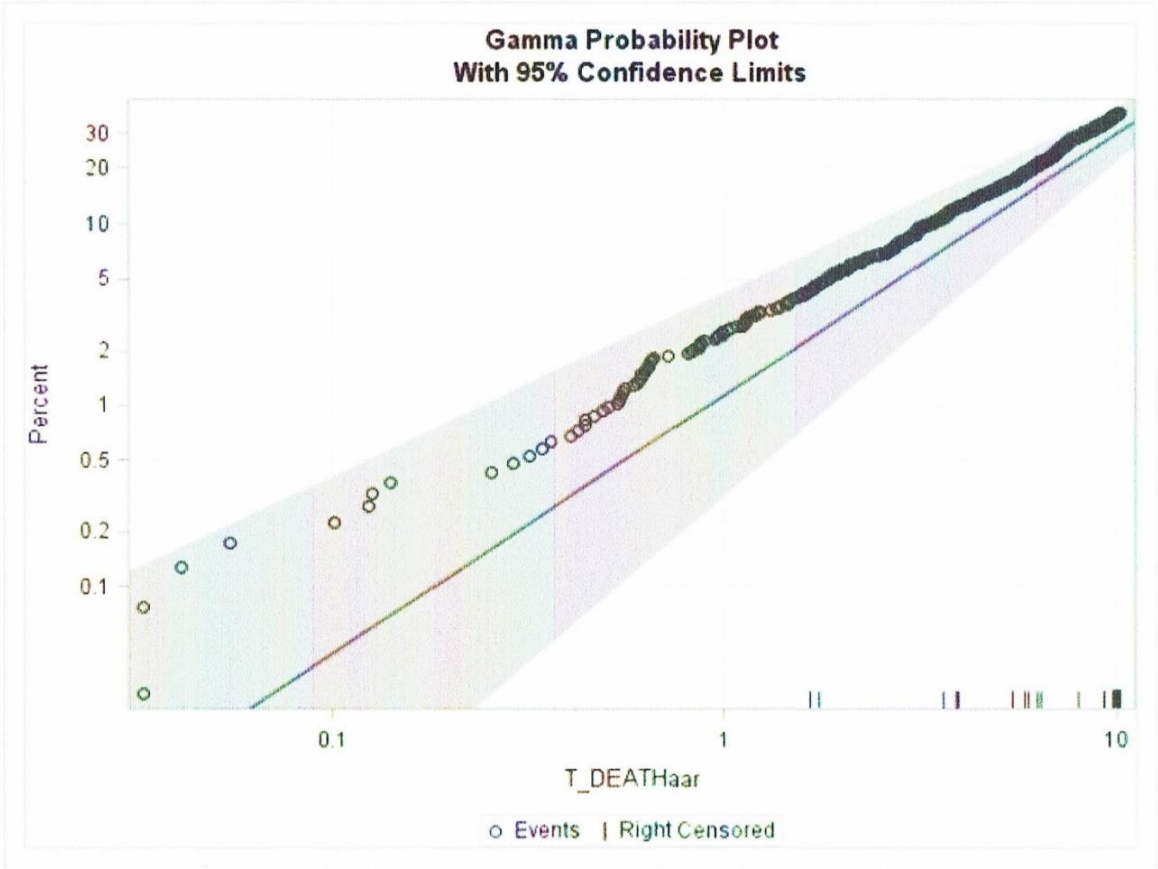

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Figure 1S B

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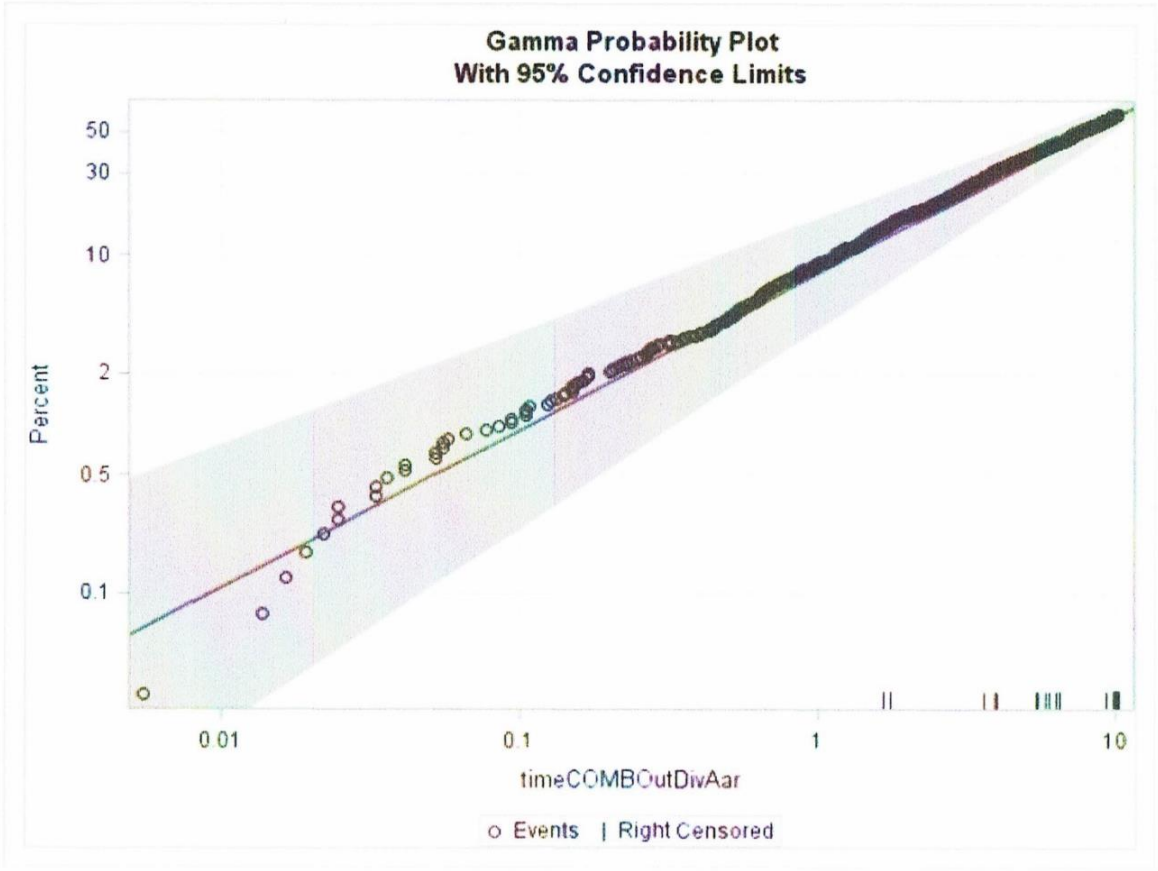

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The parametric model fitted the data reasonably well (see figure 1S A and B). The distribution of years to outcome using the accelerated failure model where the error term is modelled using the general gamma distribution showed that for both outcomes 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)	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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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.