

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Zethelius B, Berglund L, Sundström J, et al. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. *N Engl J Med* 2008;358:2107-16.

Supplementary Table 1 The crude associations of the established risk factors and biomarkers to cardiovascular and total mortality in the whole sample (n=1135)

	Cardiovascular mortality			All-cause mortality		
	Hazard ratio (95 % CI)	C-statistic	Calibration χ^2 §	Hazard ratio (95 % CI)	C-statistic	Calibration χ^2 §
Basic Model						
Age	1.23 (1.03-1.47)*	0.524	4.27	1.10 (0.98-1.24)	0.521	7.57
Systolic blood pressure	1.32 (1.12-1.55)‡	0.587‡	3.90	1.17 (1.06-1.31)†	0.543*	4.18
Anti hypertensive treatment	1.89 (1.35-2.65)‡	0.568†	-	1.34 (1.08-1.69)†	0.530*	-
Total Cholesterol	0.99 (0.84-1.18)	0.507	2.62	0.96 (0.86-1.07)	0.515	6.24
HDL-cholesterol	0.75 (0.62-0.91)†	0.591‡	3.12	0.83 (0.74-0.94)†	0.554†	4.43
Lipid Lowering treatment	1.09 (0.63-1.90)	0.500	-	0.89 (0.60-1.32)	0.507	-
Diabetes	2.35 (1.54-3.59)‡	0.553†	-	1.93 (1.44-2.60)‡	0.537‡	-
Smoking status	1.85 (1.29-2.66)‡	0.564†	-	1.64 (1.28-2.09)‡	0.546‡	-
Body mass index	1.31 (1.13-1.53)‡	0.572*	4.78	1.16 (1.04-1.99)†	0.524	6.93
Biomarkers						
Troponin I	1.67 (1.48-1.88)‡	0.637‡	0.40	1.40 (1.27-1.54)‡	0.580‡	1.34
NT-proBNP	2.05 (1.76-2.39)‡	0.706‡	1.11	1.58 (1.42-1.76)‡	0.625‡	4.21
Cystatin C	1.52 (1.33-1.74)‡	0.632‡	3.79	1.37 (1.24-1.51)‡	0.589‡	2.75
CRP	1.61 (1.36-1.91)‡	0.639‡	1.24	1.47 (1.32-1.64)‡	0.606‡	6.34

Data for continuous variables (age, systolic blood pressure, total cholesterol, HDL-cholesterol, body mass index, Troponin I, NT-proBNP, Cystatin C and CRP) is expressed as the hazard ratio for 1 standard deviation (SD) increase. * p<0.05, † p<0.01, ‡ p<0.001.

A significant value for calibration χ^2 indicates a significant deviation between predicted and observed outcomes. § The calibration χ^2 statistic is not applicable for a model with only one dichotomous predictor

Supplementary Table 2 The multivariable association of different combinations of elevated biomarkers for the prediction of cardiovascular mortality in the whole cohort (n=1135)

Biomarker combinations	Number of elevated biomarkers	Number of events /numbers at risk	Hazard ratio (95 % CI)
<u>2-biomarker combinations</u>			
Cystatin C/CRP	0	42/567	referent
	1	53/413	1.66 (1.10-2.51)*
	2	41/155	3.32 (2.11-5.22)‡
Cystatin C/Troponin I	0	53/705	referent
	1	60/381	1.99 (1.36-2.90)‡
	2	23/49	6.71 (4.00-11.23)‡
Cystatin C/NT-proBNP	0	48/699	referent
	1	56/347	2.37 (1.60-3.51)‡
	2	32/89	5.21 (3.20-8.51)‡
CRP/ Troponin I	0	53/708	referent
	1	63/378	2.22 (1.53-3.22)‡
	2	20/50	5.80 (3.39-9.90)‡
CRP/ NT-proBNP	0	46/685	referent
	1	63/376	2.39 (1.62-3.53)‡
	2	27/74	5.91 (3.60-9.70)‡
NT-proBNP/ Troponin I	0	73/904	referent
	1	38/182	2.66 (1.78-3.98)‡
	2	25/49	7.07 (4.39-11.38)‡
<u>3-biomarker combinations</u>			
Cystatin C/CRP/Troponin I	0	34/524	referent
	1	46/407	1.70 (1.08-2.67)*
	2	42/179	3.55 (2.22-5.68)‡
	3	14/25	9.83 (5.06-19.09)‡
Cystatin C/CRP/ NT-proBNP	0	30/516	referent
	1	46/403	1.99 (1.25-3.17)†
	2	40/165	3.89 (2.37-6.38)‡
	3	20/51	7.57 (4.18-13.70)‡
Cystatin C/ NT-proBNP/ Troponin I	0	41/653	referent
	1	51/349	2.31 (1.52-3.51)†
	2	26/106	4.27 (2.57-7.09)‡
	3	18/27	11.87 (6.44-21.86)‡
CRP/ NT-proBNP/ Troponin I	0	40/642	referent
	1	52/370	2.22 (1.46-3.73)‡
	2	30/98	5.12 (3.14-8.33)‡
	3	14/25	11.12 (5.84-21.17)‡
<u>4-biomarker combination</u>			
Cystatin C/CRP/Troponin I/ NT-proBNP	0	26/486	referent
	1	41/391	1.98 (1.20-3.25)†
	2	36/178	3.69 (2.19-6.20)‡
	3	22/64	7.19 (3.98-13.00)‡
	4	11/16	16.57 (7.70-35.68)‡

All models are adjusted for age at baseline (continuous), systolic blood pressure (continuous), Antihypertensive treatment (binary), Total cholesterol (continuous), HDL-cholesterol (continuous), lipid lowering treatment (binary), diabetes (binary), smoking status and body mass index (continuous). Cut-offs for biomarkers: Cystatin C ≥ 1.29 mg/l, CRP > 3 mg/l, Troponin I > 0.021 μ g/l and NT-proBNP ≥ 386 ng/l, * p<0.05, † p<0.01, ‡ p<0.001

Supplementary Table 3 The improvement in model discrimination when adding single biomarkers or different 2- and 3-combinations of the biomarkers to a model with establish risk factors for the prediction of cardiovascular mortality in participants without CVD at baseline (n=661)

		Discrimination		
		C-statistic	NRI	IDI
	Basic model	0.688	referent	referent
<u>Single biomarkers</u>	Troponin I	0.717	0.15*	0.043*
	NT-proBNP	0.722	0.12	0.044‡
	Cystatin C	0.700	-0.03	0.012
	CRP	0.715	0.10	0.013*
<u>2-biomarker combinations</u>	Cystatin C/CRP	0.714	0.05	0.020*
	Cystatin C/Troponin I	0.724	0.14	0.052*
	Cystatin C/NT-proBNP	0.724	0.15	0.046†
	CRP/ Troponin I	0.734*	0.16*	0.048*
	CRP/ NT-proBNP	0.734	0.12	0.047‡
	NT-proBNP/ Troponin I	0.737	0.21†	0.070†
<u>3-biomarker combinations</u>	Cystatin C/CRP/Troponin I	0.734*	0.15	0.056†
	Cystatin C/CRP/ NT-proBNP	0.734	0.18*	0.049‡
	Cystatin C/NT-proBNP/ Troponin I	0.739	0.20*	0.073†
	CRP/ NT-proBNP/ Troponin I	0.748*	0.24†	0.071‡

* p<0.05, † p<0.01, ‡ p<0.001, for difference with Basic model: age at baseline (continuous), systolic blood pressure (continuous), antihypertensive treatment (binary), total cholesterol (continuous), HDL-cholesterol (continuous), lipid lowering treatment (binary), diabetes (binary), smoking status (binary) and body mass index (continuous).

Discrimination -how well the model can discriminate between those who will develop the disease of interest from those who will not. The ability of a risk marker to more accurately stratify individuals into higher or lower risk categories (reclassification) is investigated by Net Reclassification Improvement (NRI, categorical) and Integrated Discrimination Improvement (IDI, continuous)

The biomarkers were modeled as continuous variables