

## Supplementary Appendix

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

Supplement to: Wang TJ, Gona P, Larson MG, et al. Multiple biomarkers for the prediction of first major cardiovascular events and death. *N Engl J Med* 2006;355:2631-9.

**Supplementary Appendix for Wang et al., “Multiple Biomarkers for the Prediction of First Major Cardiovascular Events and Death”**

- I. Description of Assay Methods
- II. Results of Multivariable Models

## I. Description of Assay Methods

Blood biomarkers were measured in morning samples collected after an overnight fast. Participants were supine for approximately 10 minutes prior to phlebotomy. Blood was immediately centrifuged, and plasma and serum were stored at  $-70^{\circ}$  C until assayed. High-sensitivity CRP was measured using a Dade Behring BN100 nephelometer. Plasma BNP and N-ANP were measured with high-sensitivity immunoradiometric assays (Shionogi, Japan).<sup>1</sup> Serum aldosterone was measured using a radioimmunoassay from extracted and fractionated serum (Quest Diagnostics, Cambridge, MA).<sup>2</sup> Plasma renin concentration was measured using an immunochemiluminometric assay (Nichols assay, Quest Diagnostics, Cambridge, MA).<sup>3</sup> Fibrinogen was measured using the method of Clauss.<sup>4</sup> D-dimer was measured using an enzyme-linked immunosorbent assay (ELISA, Biopool AB). Plasma levels of PAI-1 antigen were determined with an ELISA, following procedures described by Declerk et al (Biopool AB).<sup>5</sup> High performance liquid chromatography with fluorometric detection was used to measure total plasma homocysteine.<sup>6,7</sup> The average inter-assay coefficients of variation for each biomarker were as follows: CRP (2.2%); BNP (12.2%); N-ANP (12.7%); aldosterone (4.0% for high concentrations and 9.8% for low concentrations); renin (2.0% for high concentrations and 10.0% for low concentrations); fibrinogen (2.6%); D-dimer (11.7%); PAI-1 (7.7%); and homocysteine (9%).

The urinary albumin-to-creatinine ratio (UACR, in mg/g) was assessed on a morning urine specimen. Urinary albumin was measured using immunoturbidimetry (Tina-quant Albumin assay; Roche Diagnostics, Indianapolis, IN) and urinary creatinine was determined by the

modified Jaffe method. Coefficients of variation were 7.2% and 2.3%, respectively, for the urine albumin and urine creatinine assays.<sup>8</sup>

## II. Results of Multivariable Models

Backward elimination models were used to examine the association between multiple biomarkers and death and cardiovascular events. Results of the final models (after exclusion of biomarkers with  $p > 0.05$ ) are shown in **Appendix Table 1**. Because urine specimens were available only for a subset of participants, we performed analyses in the whole sample (9 biomarkers, omitting urinary albumin-creatinine ratio) and in the sample with urine specimens (10 biomarkers, including urinary albumin-creatinine ratio).

**Appendix Table 1: Backward elimination models for biomarker levels and incident events**

	Death				Cardiovascular Events			
	Full sample		Sample with urine specimen		Full sample		Sample with urine specimen	
<b>No. events/at risk</b>	207/3209		172/2750		169/3028		133/2598	
<b>CRP</b>	1.29 (1.10-1.50)	P=0.001	1.39 (1.19-1.63)	P<0.001	--		--	
<b>BNP</b>	--		1.40 (1.19-1.64)	P<0.001	1.35 (1.15-1.57)	P<0.001	1.25 (1.04-1.49)	P=0.02
<b>N-ANP</b>	1.39 (1.17-1.65)	P<0.001	--		--		--	
<b>Aldosterone</b>	--		--		--		--	
<b>Renin</b>	1.14 (1.00-1.30)	P=0.04	1.17 (1.01-1.35)	P=0.03	--		--	
<b>Fibrinogen</b>	--		--		--		--	
<b>D-dimer</b>	1.24 (1.02-1.50)	P=0.03	--		--		--	
<b>PAI-1</b>	--		--		1.24 (1.02-1.51)	P=0.03	--	
<b>Homocysteine</b>	1.22 (1.06-1.40)	P=0.006	1.20 (1.03-1.41)	P=0.02	--		--	
<b>UACR</b>	n/a		1.22 (1.06-1.41)	P=0.007	n/a		1.20 (1.02-1.41)	P=0.03

Values shown are multivariable-adjusted hazard ratios, with 95% confidence interval in parentheses. Hazard ratios correspond to a 1 standard deviation increment in the natural logarithm of the biomarker value. Only hazard ratios for biomarkers retained in backward elimination models are shown, using p<0.05 as the threshold for retention. All hazard ratios were adjusted for age, sex, body-mass index, total cholesterol category, HDL cholesterol category, cigarette smoking, blood pressure category, diabetes mellitus, serum creatinine, and prevalent cardiovascular disease (in model for death). Abbreviations: BNP, B-type natriuretic peptide; CRP, C-reactive protein; N-ANP, N-terminal pro-atrial natriuretic peptide; PAI-1, plasminogen activator inhibitor-1; UACR, urinary albumin-creatinine ratio.

## References for Appendix

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