SUPPLEMENTAL MATERIAL

Supplemental Methods

Data S1. The principle of linear mixed-effects modelling

Linear mixed-effect models extend simple linear models by considering both fixed effects and random effects. The simplest linear regression model includes an intercept parameter and a slope parameter. The estimated intercept represents, for example, the expected biomarker concentration at baseline. The estimated slope represents the expected change in biomarker concentration per month (i.e., rate of change). Both the intercept and slope parameter are considered "fixed" effects, meaning that every individual in the population has the same expected value (i.e., mean) for the intercept and slope. A linear mixed-effect model does not only consider fixed effects, but also includes random effects that capture individual variations around the fixed (population average) effects. Linear mixed-effects models are particularly used when there is non-independence in the data, like repeated measurements within individuals. For instance, a random intercept may be used in a repeated measures context to account for variation between individuals in their baseline biomarker levels. The model would still contain a fixed intercept, representing the mean intercept for all individuals, but each individual then also gets their own estimated random effect, representing an adjustment from the mean that is unique to the individual. In other words, each person may have their own baseline biomarker level (random intercept), unique to them, while still sharing a common overall baseline (fixed intercept). The fixed effects in combination with the random effects describe an individual's biomarker trajectory. One of the advantages of a linear mixed-effect model is that it allows us to describe both average population trajectories (i.e., marginal trajectories) as well as subject-specific trajectories (i.e., individual trajectories).

In the current study, linear mixed-effects modeling was applied to assess the subject-specific biomarker trajectories in separate, unadjusted single biomarker models using the nlme package. More specifically, biomarker concentrations were used as dependent variables, while sampling time during the follow-up served as the independent variable and was included as a fixed effect. To allow biomarker concentrations to differ between individuals at baseline, a random intercept was included as random effect in the linear mixed-effect models. Additionally, to allow the biomarker trajectories between individuals to vary over time, sampling time during follow-up was also entered as a random effect in our models, specifically as random slopes. These random effects in combination with the fixed effects describe an individual's biomarker trajectory and enabled us to estimate the individual patients' biomarker concentrations at 30 days after the index ACS, as well as the rate of change during the 30-days to 1-year post-ACS period.

Table S1. Associations between clusters based on single 1-year biomarker estimates and long-term mortality.

	Model 1: unadjusted		**Model 2: adjusted for age**		**Model 3: adjusted for age and sex**		**Model 4: adjusted for age, sex and CVD risk factors****	
	STR (95% CI)*	**P-value**	**STR (95% CI)***	**P-value**	**STR (95% CI)***	**P-value**	**STR (95% CI)***	**P-value**
'Low' biomarker cluster	-ref-		-ref-		-ref-		-ref-	
'High' biomarker cluster	0.26 (0.18, 0.38)	**<0.001**	0.50 (0.35, 0.73)	**<0.001**	0.51 (0.35, 0.73)	**<0.001**	0.56 (0.39, 0.82)	**0.003**
Re-ACS cluster	0.37 (0.19, 0.69)	**0.002**	0.58 (0.31, 1.09)	0.089	0.60 (0.32,1.11)	0.104	0.70 (0.37, 1.31)	0.261
Re-ACS cluster	-ref-		-ref-		-ref-		-ref-	
'High' biomarker cluster	0.72 (0.40, 1.29)	0.265	0.85 (0.48, 1.50)	0.316	0.84 (0.48, 1.47)	0.530	0.76 (0.43, 1.32)	0.327

* Survival Time Ratio (STR) can be interpreted as the average survival time since the 1-year landmark of one cluster relative to a reference cluster.

** Diabetes, hypertension, hypercholesterolemia, smoking status, body mass index, diagnosis of index ACS, history of coronary artery disease, history of stroke, and estimated glomerular filtration rate.

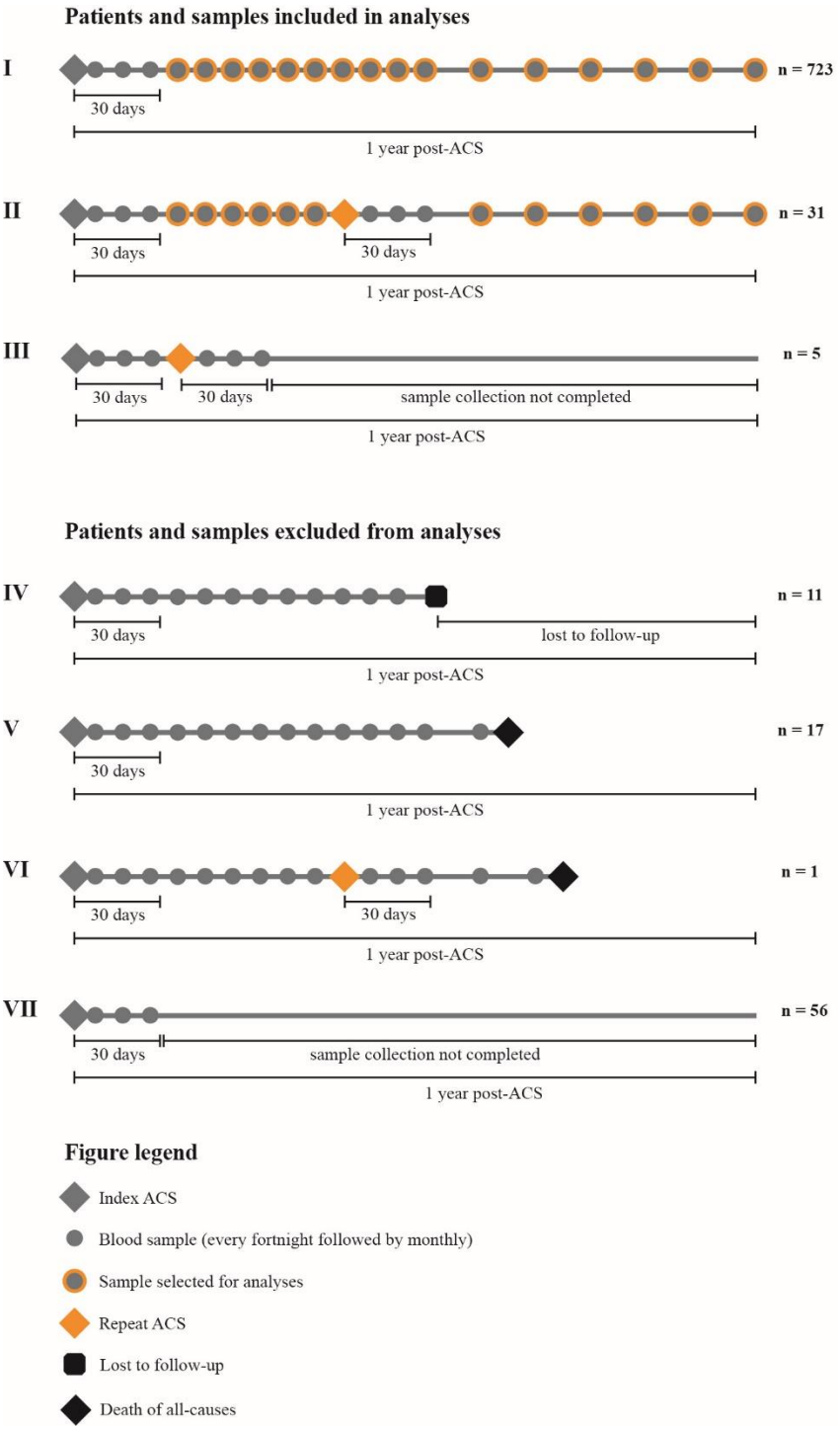

Figure S1. Patient and sample collection.

The current study included the 723 patients who were known to be alive one year after the index event, who had at least one biomarker measurement available, and who did not experience a repeat ACS during the first year after the index event, as well as 36 patients who did experience a (non-fatal) repeat ACS. Cluster analysis was performed on repeated biomarker data of the 723 individuals who remained event-free during the first year following ACS (Situation I), whereas those who experienced a re-ACS in the first year after the index event were considered a separate, re-ACS cluster (Situation II and III). No biomarker measurements were available for 5 of these re-ACS patients (Situation III) and, consequently, these patients were not include in the biomarker trajectory plots (Figure 1 and Supplemental Figure S3). In the current study, patients were excluded from the cluster analysis if data on vital status 1-year after the index ACS was missing (Situation IV), if patients did not survive the first year after the index ACS (Situation V and VI), or if there were no samples available >30 days after the index ACS (Situation VII). In total, the current study is based on the 10,606 samples (median of 16 samples per patient) that were obtained >30 days after the index ACS and that were not obtained in the 0 to 30-day window following a re-ACS.

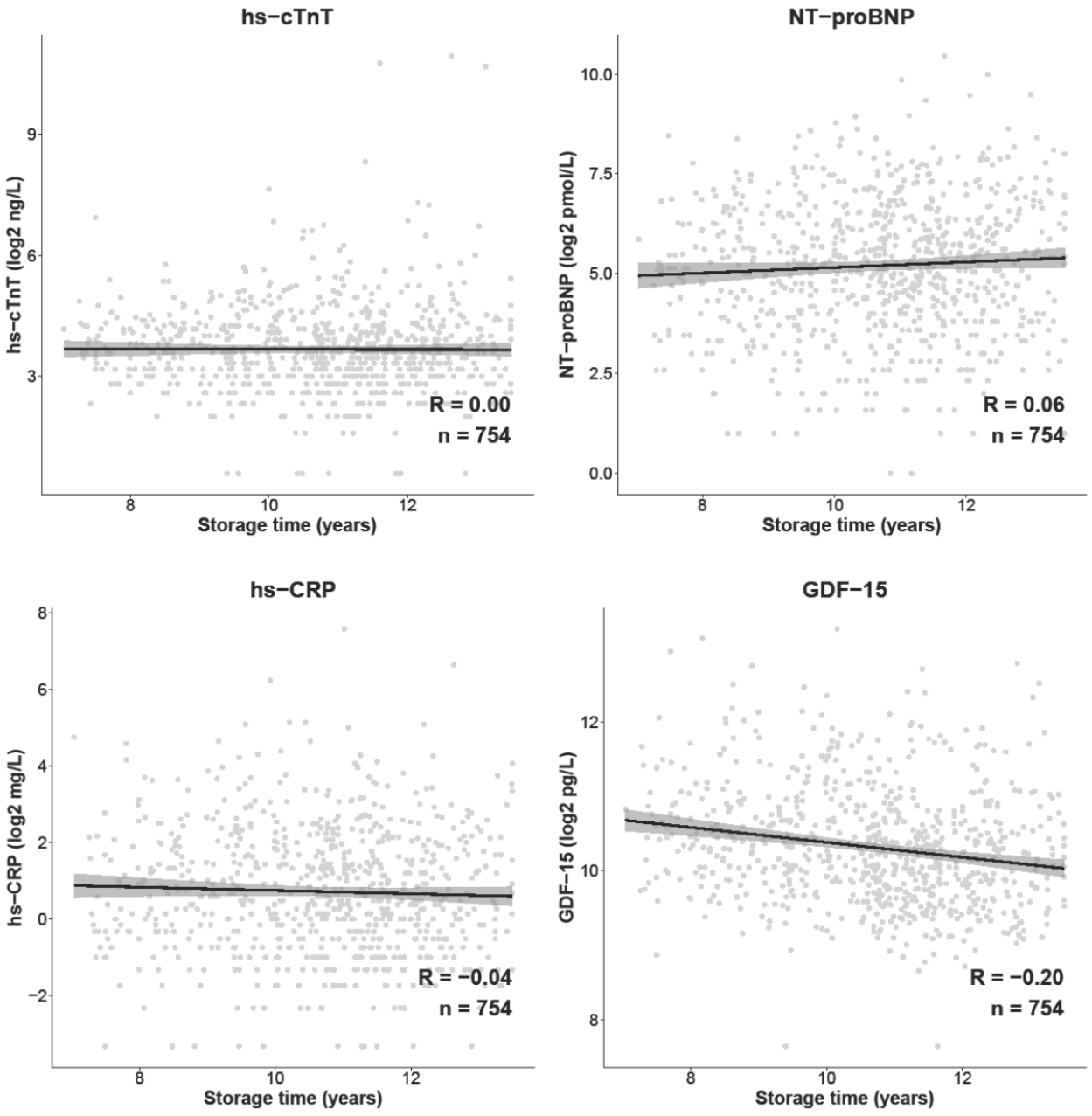

Figure S2. Correlation between individuals' biomarker concentrations 30 days after the index ACS and storage duration.

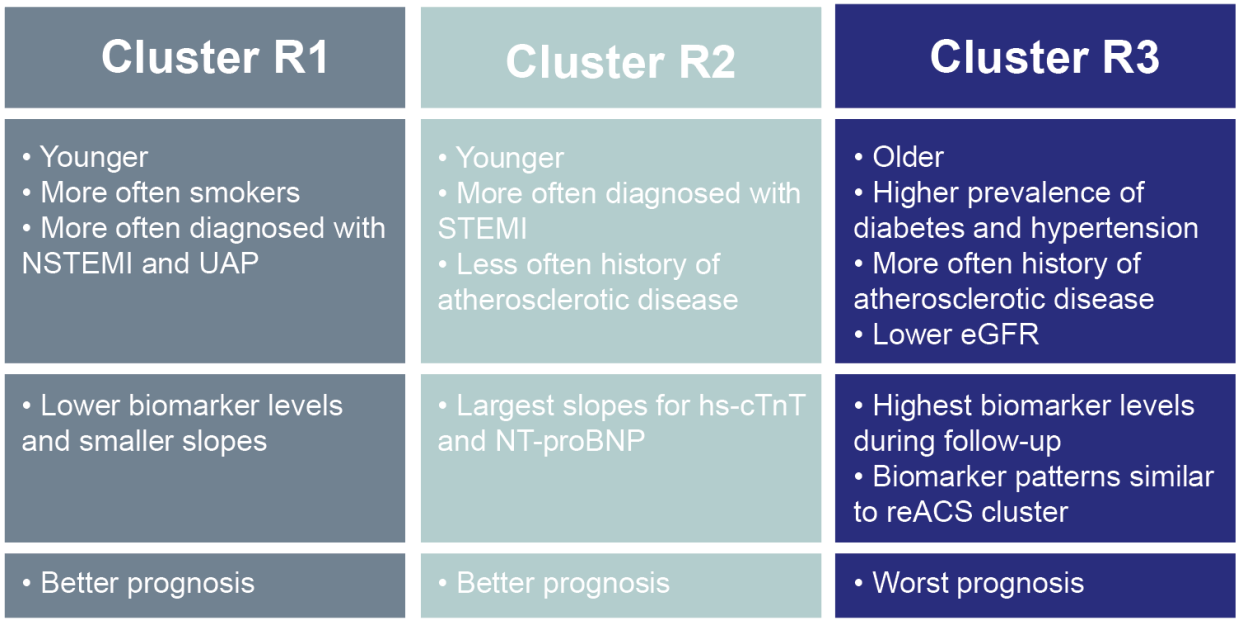

Figure S3. Overview of repeated measurement based cluster characteristics.

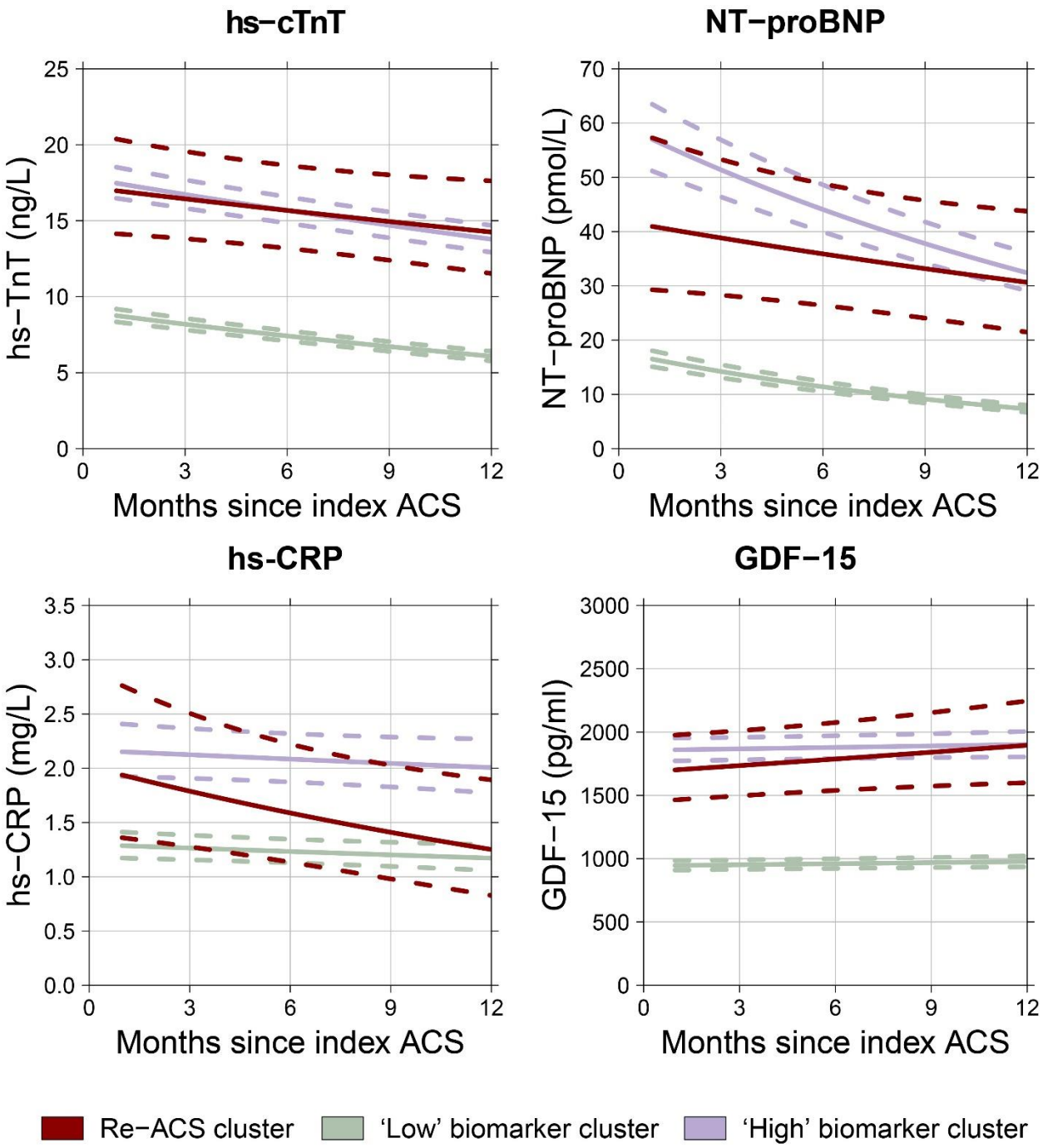

Figure S4. Temporal trajectories of clusters based on single 1-year biomarker estimates.

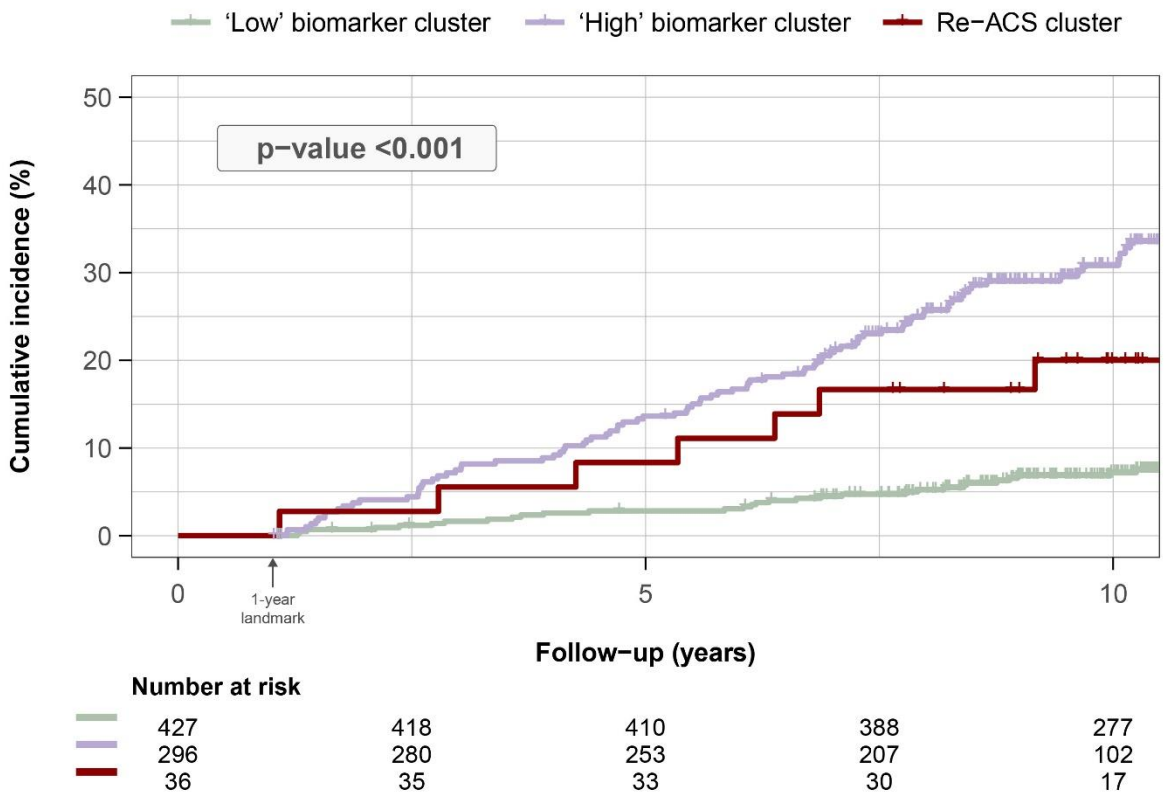

Figure S5. Association between clusters based on single 1-year biomarker estimates and long-term mortality.