SUPPLEMENTARY MATERIAL

Prognostic value of C-reactive protein in adults with congenital heart disease

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Supplementary File 1. Method for assessing the variability of repeatedly measured hs-CRP in adults with congenital heart disease.

The variability of hs-CRP was investigated in the subset of the patients that remained free of the primary endpoint en who had at least 2 hs-CRP measurements (n= 495). We first calculated the coefficient of variation (CV) as follows;

$$\mathrm{CV} = 100\% * \mathrm{sd}/\overline{\mathrm{X}}$$

Thereafter, the method described by Fraser and Harris[1] to assess the variation of a set of repeated measurements was used to further investigate the variability of hs-CRP measurements. According to this method, the total variation can be distinguished into three components; the analytical variation ($\mathrm{CV_a}$) due to the analytical measurement imprecision, the within-subject variation ($\mathrm{CV_i}$), and the between-subject variation ($\mathrm{CV_g}$). The $\mathrm{CV_a}$ of hs-CRP in our laboratory was estimated at 12% and 8.6% for low and high concentrations, respectively.

$$\mathrm{CV_i} = \mathrm{median}\sqrt{(\mathrm{CV}^2_{\mathrm{subject}}) - \mathrm{CV}^2_{\mathrm{a}}}$$

$$\mathrm{CV_g} = 100\% * \mathrm{sd}_{\overline{\mathrm{X}}_{\mathrm{subject}}}/\overline{\mathrm{X}}_{\mathrm{group}}$$

The index of individuality (II) was calculated accordingly:

$$\mathrm{II} = \sqrt{\mathrm{CV}^2_{\mathrm{i}} + \mathrm{CV}^2_{\mathrm{a}}}/\mathrm{CV_g}$$

Lastly, the reference change value (RCV) was calculated. Because hs-CRP was not normally distributed, the lognormal approach was used to calculate the RCV limits[2]. We used $\alpha = 0.05$ (95% confidence), corresponding to $\mathrm{Z}_{0.025} = 1.96$.

$$\mathrm{RCV} = \mathrm{Z}_{\alpha/2} * \sqrt{2(\mathrm{CV}^2_{\mathrm{i}} + \mathrm{CV}^2_{\mathrm{a}})}$$

$$\mathrm{RCV_{low}} = \mathrm{e}^{-\mathrm{Z}_{\alpha/2}*\sqrt{2\ln(\mathrm{CV}^2_{\mathrm{w}}+\mathrm{CV}^2_{\mathrm{a}}+1)}} - 1$$

$$\mathrm{RCV_{up}} = \mathrm{e}^{\mathrm{Z}_{\alpha/2}*\sqrt{2\ln(\mathrm{CV}^2_{\mathrm{w}}+\mathrm{CV}^2_{\mathrm{a}}+1)}} - 1$$

References:

1 Fraser CG, Harris EK. Generation and application of data on biological variation in clinical chemistry. *Crit Rev Clin Lab Sci* 1989;**27**:409-37.

2 Fokkema MR, Herrmann Z, Muskiet FA, et al. Reference change values for brain natriuretic peptides revisited. *Clin Chem* 2006;**52**:1602-3.

Supplementary File 2. Specification of the linear mixed effects model.

All variables considered relevant to describe the evolution of hs-CRP over time were included as fixed effects. The fixed effects consisted of repeated hs-CRP, time, diagnosis, sex, age, NYHA class, cardiac medication use, rhythm, systemic ventricular function, body mass index and eGFR. Interactions of time with diagnosis and sex was considered but did not improve model fit. Also, a non-linear term for age was considered using a natural cubic spline with 3 degrees of freedom, but did not improve the model and was therefore left out of the model. The random effects consisted of a non-linear random slope (cubic spline with 3 degrees of freedom) and a random intercept.

Supplementary File 3. Rationale and assumptions of the Joint Model.

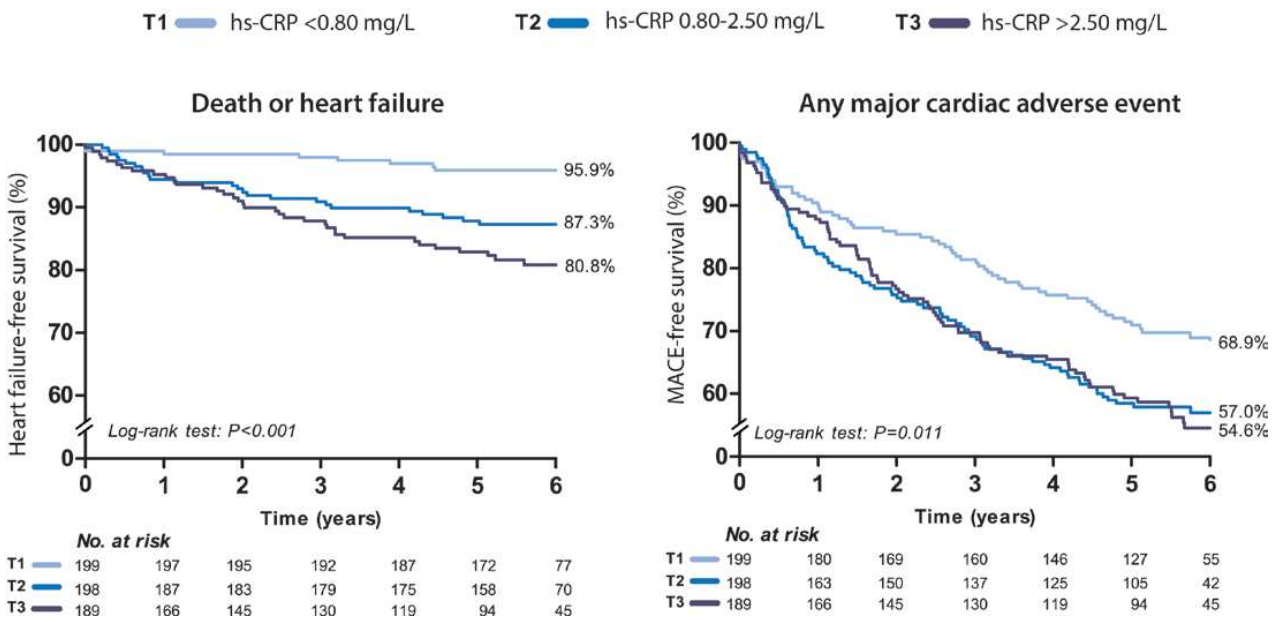
The rationale behind the use of Joint Model is based on the fact that blood biomarkers are so-called endogenous time-dependent covariates; they vary dynamically over time, and are directly related to a subject and thus depending on other variables. In addition, the availability of blood biomarkers is related to the survival status of the patient. To account for the special features of these covariates, the use of a Joint Model is recommended for longitudinal and time-to-event data.[1] The concept of a Joint Model is that both models are estimated jointly in such way that the (modelled) value of the longitudinal marker at each point in time is related to the hazard of the endpoint event. One of the features is that the level of the longitudinal marker is not assumed constant between two subsequent visits in this framework.

The key assumption of a Joint Model is Full Conditional Independence. The validity of this assumption depends on whether the censoring and planning of visits is non-informative. This means that patients decision to withdraw from the study or the patient's appearance for the next study visit may depend on observed history of the patients (baseline covariates and observed longitudinal measures), however it may not depend on underlying latent patient characteristics associated with prognosis. There is no statistical test to validate this assumption, but its validity is based on the design of the study. Likewise Linear Mixed Effects models, the residuals of the Joint Model should be approximately normally distributed. After fitting each Joint Model, normality of residuals were checked by QQ-plots. No relevant deviation of normality was observed.

Reference:

1 Rizopoulos D, Takkenberg JJ. Tools & techniques--statistics: Dealing with time-varying covariates in survival analysis--joint models versus Cox models. *EuroIntervention* 2014;**10**:285-8.

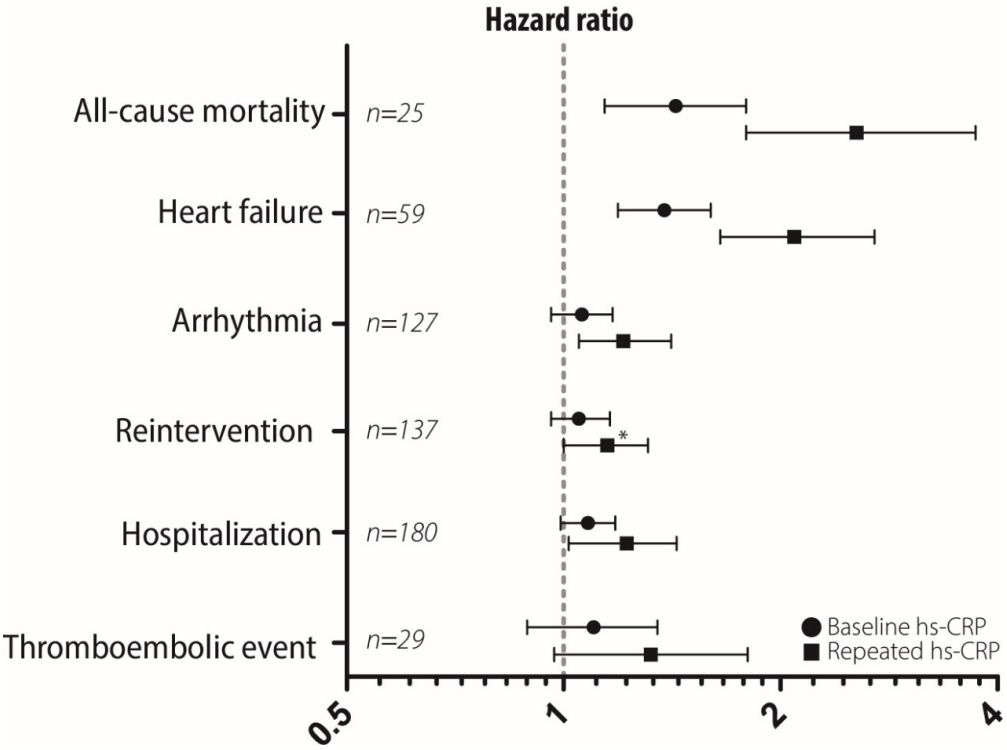
Supplementary File 4. Survival according to the tertile hs-CRP distribution for the primary (death or heart failure) and secondary endpoint (any major cardiac adverse event) in adults with congenital heart disease.



Legend: Survival curves were compard with the log-rankt test for trend.

Abbrevations: hs-CRP= high-sensitivity C-reactive protein, T1=tertile 1, T2= tertile 2, T3= tertile 3

Supplementary File 5. Univariable associations of baseline and repeated hs-CRP with the individual event components of the endpoints in adults with congenital heart disease.



Legend: *p-value <0.05. Hazard ratios are expressed per two-fold higher CRP level. Occurrence of the number of each individual endpoints reached in the study is given for each event.

Abbreviations: hs-CRP= high-sensitivity C-reactive protein

Supplementary File 6. Subanalysis in patients with an elevated NT-proBNP >14 pmol/L (n=312) for the association between hs-CRP and all-cause mortality or heart failure.

	Death or heart failure	
	HR (95%CI)	**p-value**
Baseline hs-CRP	1.22 (1.06-1.42)	0.007
Adjusted for baseline characteristics*	1.16 (1.01-1.34)	0.035
Adjusted for baseline NT-proBNP	1.18 (1.02-1.35)	0.026
Adjusted for baseline characteristics* and baseline NT-proBNP	1.16 (1.01-1.33)	0.034
Repeated hs-CRP *(unadjusted)*	1.69 (1.36-2.11)	<0.001
Adjusted for baseline characteristics*	1.53 (1.20-1.98)	<0.001
Adjusted for baseline NT-proBNP	1.58 (1.26-2.00)	<0.001
Adjusted for baseline characteristics* and baseline NT-proBNP	1.48 (1.18-1.89)	<0.001
Repeated hs-CRP and NT-proBNP		
Repeated hs-CRP	1.51 (1.18-1.94)	<0.001
Repeated NT-proBNP	2.29 (1.90-2.75)	<0.001

Legend: *age, sex, congenital diagnosis, NYHA, cardiac medication, systemic ventricular function. The endpoint of death or heart failure was met in 67 (21.4%) patients. HRs are expressed per two-fold higher biomarker level.

Abbreviations: hs-CRP= high-sensitivity C-reactive protein, HR=hazard ratio, NT-proBNP= N-terminal pro B-type natriuretic peptide