Supplemental Digital Content

Systemic Inflammation after Critical Illness: Relationship with Physical Recovery, and Exploration of Potential Mechanisms

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1. HCMV sero-positivity sensitivity analysis

2. Missing data sensitivity analysis

1. HCMV sero-positivity analysis

We conducted a sensitivity analysis to test whether HCMV sero-positivity altered the relationship between CRP and RMI in the multivariable model described in table 4 in the main manuscript. The results are presented below (eTable1).

	Co-efficients	B	Lower 95% CI	Upper 95% CI	p
Model 1	Ventilator days*	0.05	-0.13	0.23	0.58
	Age	0.00	-0.01	0.01	0.71
	Gender	0.11	-0.16	0.37	0.43
	APACHE 2	0.01	-0.01	0.03	0.49
	FCI	0.10	0.03	0.16	<0.01
	CRP*	0.14	0.06	0.23	<0.01
Model 2	Ventilator days*	0.07	-0.12	0.25	0.49
	Age	-0.00	-0.01	0.01	0.83
	Gender	0.10	-0.18	0.38	0.47
	APACHE 2	0.01	-0.01	0.02	0.59
	HCMV sero-positivity	0.09	-0.19	0.37	0.52
	FCI	0.10	0.04	0.17	<0.01
	CRP*	0.15	0.06	0.24	<0.01

eTable 1: Multivariable model exploring the association of CRP concentration with Rivermead mobility index** adjusted for the confounders of age, gender, ventilator days, apache 2 score, and functional comorbidity index (model 1). Model 2 shows the influence of adding HCMV sero-positivity to this model. *Natural log transformed variables. RMI was inverse natural log transformed (ln(16-RMI)).

The relationship between HCMV sero-positivity and RMI was not significant and did not alter the relationship between CRP and RMI.

2. Missing Data

The exploration of the relationship between systemic inflammation and physical recovery was subject to missing data, particularly in the biomarker data fields. Data loss occurred due to death, failed venepuncture, patient refusal of blood sampling, loss to follow up, and withdrawal. A very small number of data points were lost due to an inadequate volume of sample available for a particular assay.

Approaches to missing data in epidemiological studies has been standardized (1) and are influenced by the suspected mechanism of the missing data. Missing data can be classified as missing completely at random (MCAR), in which case a complete case analysis may be appropriate; missing at random (MAR) where the values can be predicted from other variables in the study database and methods such as multiple imputation (MI) might be appropriate, and missing not at random (MNAR), where the values of the missing data are related to values of the missing data. There exists no statistical method that can distinguish MCAR from MAR because the true values of the missing data can never be known, but based on knowledge of the study conduct one can hypothesise about the likely underlying mechanism.

Based on the above observations, a missing at random (MAR) mechanism was considered most likely, but a missing not at random (MNAR) mechanism could not be excluded. We tested whether adopting alternative techniques to adjust our analysis for these assumptions would lead to a departure in the effect size precision calculated using the complete case approach that we adopted in our main analysis. A sensitivity analysis based on our main analysis between CRP and RMI adjusted for the potential confounding factors of age, gender, APACHE 2 score, functional comorbidity index, and days of mechanical ventilation) was conducted.

Complete case analysis

We present the analysis that was used in the manuscript. Complete cases were considered representative of the cases with missing values.

MAR assumption

For the MAR assumption, the multiple imputation function in the statistical software package SPSS (IBM SPSS Statistics version 21) was used to impute missing variables in all fields which uses a Monte Carlo chained equations methodology. A pooled analysis of one-hundred imputed datasets were performed. Non-normal variables were transformed prior to imputation.

MNAR assumption

For the MNAR assumption, CRP values were assumed to be higher in those patients with missing data in this field. For this reason, natural log transformed CRP was set to zero, and all other values were imputed using the method used above.

The sensitivity analysis is presented in eTable 2. The relationship between CRP and RMI did not change significantly between the models – and was found to be statistically significant regardless of which method was used to deal with missing data. We therefore adopted a complete case approach for all other analyses.

	Co-efficients	B	Lower 95% CI	Upper 95% CI	p
Complete Case	Ventilator days*	0.05	-0.13	0.23	0.58
	Age	0.00	-0.01	0.01	0.71
	Gender	0.11	-0.16	0.37	0.43
	APACHE 2	0.01	-0.01	0.03	0.49
	FCI	0.10	0.03	0.16	<0.01
	CRP*	0.14	0.06	0.23	<0.01
All variables multiply imputed (MAR assumption)	Ventilator days*	0.08	-0.07	0.23	0.31
	Age	0.01	-0.00	0.02	0.10
	Gender	0.16	-0.07	0.39	0.18
	APACHE 2	0.00	-0.01	0.02	0.73
	FCI	0.06	0.01	0.12	0.03
	CRP*	0.09	0.00	0.18	0.04
ln(CRP) set to 0 (MNAR assumption), all other missing variables multiply imputed	Ventilator days*	0.08	-0.07	0.23	0.30
	Age	0.01	-0.00	0.02	0.10
	Gender	0.14	-0.09	0.37	0.22
	APACHE 2	0.00	-0.02	0.02	0.83
	FCI	0.06	0.00	0.12	0.04
	CRP*	0.08	0.00	0.16	0.04

eTable 2 – sensitivity analysis showing effect of various missing data assumptions. MAR: missing at random; MNAR: missing not at random. Outcome variable was ln(16-RMI). *Ventilator days and CRP were natural log transformed.

1. Sterne JAC, White IR, Carlin JB, Spratt M, Royston P, Kenward MG, et al. Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. BMJ. 2009 Jun 29;338:b2393.