Title: C-reactive protein prior to myeloablative allogeneic haematopoietic cell transplantation identifies patients at risk of early and long term mortality

Runner: Pre-allograft C-reactive protein identifies patient mortality

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## Keywords

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Patient selection for allogeneic haematopoietic cell transplantation (HCT) is complex and outcome can be predicted by the HCT comorbidity index (HCT-CI) ([Sorror, et al 2005](#_ENREF_6)) or the European Group for Blood and Marrow Transplantation (EBMT) score ([Gratwohl 2012](#_ENREF_3), [Gratwohl, et al 1998](#_ENREF_4)). HCT-CI accounts for patient factors such as proven or suspected recipient infection, and the EBMT score includes disease and treatment factors. Pre-myeloablative (MAC) and reduced intensity (RIC) conditioning C-reactive protein (CRP) level is a promising routine inexpensive and internationally available biomarker that may be associated with clinical outcomes post allogeneic HCT ([Aki*, et al* 2012](#_ENREF_1), [Artz*, et al* 2008](#_ENREF_2), [Pavlu*, et al* 2010](#_ENREF_5)). This study addresses whether CRP elevation above a normal reference range (>9 mg/l) prior to MAC is additive to information provided by the HCT-CI and component factors of the EMBT score, in identifying patients with inferior early (NRM at 100 days) or late survival at five years, compared to patients with a normal CRP (0−9 mg/l).

A retrospective cohort study using prospectively acquired routine clinical data was undertaken. Primary endpoints were: survival calculated from the day of HCT until death from any cause; and NRM, defined as any cause of death not related to relapse that occurred within 100 days post HCT. The secondary outcomes were disease relapse, acute graft versus host disease (GvHD), and chronic GvHD. Further details are presented in the online supplement.

Two hundred and sixty four adults were identified that received MAC prior to allogeneic HCT over the eighteen year study period. Eleven patients with incomplete CRP data were excluded (online supplement, Fig 1). The median age of the 253 evaluable patients was 37 years (range 17–63). Indications for transplant are outlined in the online supplement, (Table S1).

## The primary outcome of NRM at 100 days was 19.8% (95% confidence interval (CI) 16–25) and survival at five years was 41.9% (95% CI 35–47), with a median follow up of 60.6 months (range 3–187) for patients alive at last contact. Validation of the HCT-CI and EBMT score, and univariate analyses with CRP are presented in the online supplement (Tables S2-S3).

CRP is associated with NRM independent to the HCT-CI and EBMT score components**.** In a multivariate regression model for the outcome of NRM, an elevated CRP >9 mg/l was associated with a HR of 2.47 (95% CI 1.4–4.4; *P* = 0.002) compared to a normal CRP of 0–9 mg/l. Patient comorbidity pre-defined by a HCT-CI of ≥3 was also independently associated with NRM (HR 2.49; 95% CI 1.2–5.3; P = 0.014). The only other covariates independently associated with NRM were advanced disease stage (HR 2.31; 95% CI 1.3–4.3; P = 0.007) and patient CMV IgG serology (HR 2.39; 95% CI 1.4–4.5; P = 0.007). The aggregated EBMT score or other component covariates were not retained in the model (Table 1).

CRP is associated with survival to five years independent to the HCT-CI and EBMT score components. The multivariate regression model for survival found that an elevated CRP of >9 mg/l was associated with a HR of 1.95 (95% CI 1.4–2.7; P < 0.001) compared to a normal CRP of 0–9 mg/l. Patient comorbidity pre-defined by a HCT-CI of ≥3 was also independently associated with survival at five years (HR 2.49; 95% CI 1.2–5.3; P = 0.014). The only other covariates independently associated with survival were advanced disease stage (HR 1.94; 95% CI 1.3–2.9; P = 0.011) and treatment from 2002 onwards (HR 0.54; 95% CI 0.4–0.8; P = 0.001). The aggregated EBMT score or other component covariates were not retained in the model (Table 1).

CRP is independently associated with NRM and survival in patients at low risk of death by the HCT-CI.Patients at low risk of NRM at 100 days and mortality at five years can already be identified with a low HCT-CI of 0–2, which was retained along with CRP in multivariate models presented in this study (Table 1). In a predefined 183 patient subgroup with a HCT-CI 0–2, NRM was 10.3% (95% CI 9–12), and survival was 39.2% (95% CI 32–45). An elevated CRP level remained independently associated with NRM (HR 2.21; 95% CI 1.1–4.7; P = 0.037) and survival (HR 2.0; 95% CI 1.3–3.0; P = 0.001) in this low risk subgroup in multivariate models (data not shown).

To investigate whether CRP might be a biomarker of occult infection pre-transplantation that results in early or late mortality caused by sepsis, we analysed whether an elevated CRP was associated with a particular cause of death (online supplement, Table S4). Although 57% of NRM and 39% of late mortality to five years were attributed to infections causing severe sepsis or septic shock, an elevated CRP of >9 mg/l was not associated with these, or any other specific cause of death. Thus, CRP is not associated with early or late mortality from sepsis.

The secondary outcome of relapse probability at five years for the entire cohort was 29.6% (95% CI 24–36%), the incidence of acute GvHD grades 2–4 was 51% (129 out of 234 patients), and the probability of any chronic GvHD at five years was 60.0% (95% CI 53-68%). Relapse risk, acute GvHD, and chronic GvHD, were not associated with the HCT-CI, the aggregated EBMT score, or CRP (data not shown).

We conclude by reporting for the first time that an elevated CRP level prior to MAC allogeneic HCT is associated with NRM at 100 days and survival at five years either alone or when combined with the HCT-CI, as well as components of the EBMT score. The independent association of an elevated CRP >9 mg/l was robust even in patients identified as low risk by the HCT-CI. We confirm that an elevated CRP is a valid and robust pre-MAC biomarker associated with inferior early and late survival post HCT, and should be integrated into existing risk assessment tools when considering treatment of patients with allogeneic HCT.

# Competing interests

The authors have no competing interests.

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# Author contributions

AP, RS and JP planned the study, performed the analysis, and wrote the manuscript, with input from all the authors. AP, RS, JP, and HWA collected the data. All authors developed the final manuscript, and had full access to all of the data.

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# Tables

Table 1. Multivariate regression models for the primary outcomes of non-relapse mortality (NRM) at 100 days post transplant and survival at five years. The hazard ratio (HR) and 95% confidence intervals (CI) are presented. Abbreviations: C-reactive protein (CRP); haematopoietic cell transplantation comorbidity index (HCT-CI); European Group for Blood and Marrow Transplantation (EBMT); CMV (cytomegalovirus); number (N).

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|  | NRM at 100 days | Survival at five years |
| Covariate factor | **Patients (N)** | **HR****(95% CI)** | **p-value** | **Patients (N)** | **HR** **(95% CI)** | **p-value** |
| CRP level 0-9 mg/l >9 mg/l | 15978 | 1.02.47 (1.4-4.4) | 0.002 | 16379 | 1.01.95 (1.4-2.7) | <0.001 |
| HCT-CI 0-2 ≥3 | 17958 | 1.02.49 (1.2-5.3) | 0.014 | 18359 | 1.01.64 (1.1-2.4) | 0.011 |
| EBMT disease stage Early or Intermediate Late | 19542 | 1.02.31 (1.3-4.3) | 0.007 | 19943 | 1.01.94 (1.3-2.9) | 0.001 |
| Recipient CMV IgG Not detected Detected | 100137 | 1.02.39 (1.4-4.5) | 0.007 | Not retained within the survival model |
| Transplant decade 1993-2001 2002-2011 | Not retained within the NRM model | 127115 | 1.00.54 (0.4-0.8) | 0.001 |

# Figures

Fig 1. Probability of non-relapse mortality (NRM) at 100 days and survival to five years stratified by haematopoietic cell transplantation (HCT) comorbidity index (HCT-CI; 0-2 and ≥3; A and C) and pre-myeloablative conditioning C-reactive protein (CRP) levels (0–9 mg/l and >9 mg/l; B and D).