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

##### Amit Patel 1,2, Richard M Szydlo 2, Holger W Auner 2, Edward J Kanfer 3, Donald H MacDonald 2,3, Dragana Milojkovic 2,3, Syed Altaf 3, Andrew Innes 3, Ian Gabriel 3,4, Amin Rahemtulla 3, Aristeidis Chaidos 3, Anastasios Karadimitris 2, Eduardo Olavarria 3, Jane F Apperley 2,3, and Jiri Pavlu 2,3

1 Targeted Therapy, Division of Cancer Biology, Institute of Cancer Research, London, UK

2 Centre for Haematology, Faculty of Medicine, Hammersmith Hospital, Imperial College London, London, UK

3 Centre for Haematology, Hammersmith Hospital, Imperial College Healthcare NHS Foundation Trust, London, UK

4 Department of Haematology, Chelsea and Westminster Hospital, Chelsea and Westminster Hospital NHS Foundation Trust, London, UK

Correspondence:

Dr Jiri Pavlu, MUDr, MD, MRCP, FRCPath

Room 2.20 Catherine Lewis Centre

Hammersmith Hospital, Du Cane Road, London, W12 0HS.

T. +44(0)-20-8383-8117/5030

F. +44(0)-20-8383-3965

# Summary

There is unmet clinical need to improve patient selection for allogeneic haematopoietic cell transplantation (HCT). In a 253 patient study we tested whether an elevated C-reactive protein (CRP) (>9 mg/l) prior to myeloablative conditioning identified patients with excess non-relapse mortality (NRM) at 100 days and survival at five years. An elevated CRP was independently associated with inferior NRM (hazard ratio (HR) 2.47 (95% CI 1.4-4.4), *P* = 0.002)) and survival (HR 1.95 (95% CI 1.4-2.7), *P* < 0.001)) using adjusted models incorporating existing comorbidity and disease risk assessment tools. CRP should be integrated in to HCT patient selection decisions.

# Methods, additional details

## Study design and definitions

Endpoints were defined according to EBMT guidelines ([Iacobelli and Committee 2013](#_ENREF_9)). Relapse rate was defined as recurrence of the disease indication for allogeneic HCT; acute GvHD as onset of any grade ≤100 days post allogeneic HCT, and chronic GvHD after this time point (Iacobelli and Committee 2013). Causes of death were categorised as GvHD, disease progression or relapse (Iacobelli and Committee 2013), severe sepsis or septic shock due to infections (Bone, et al 2009), and others.

We used the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) ([von Elm, et al 2007](#_ENREF_16)) guidelines and checklist, and the CONSORT (Consolidated Standards of Reporting Trials) ([Moher, et al 2010](#_ENREF_11)) flow diagram. All patients were consented for non-interventional research. The study was conducted in accordance with the Ethical Principles for Medical Research Involving Human Subjects outlined in the Declaration of Helsinki (http://www.wma.net/en/30publications/10policies/b3/index.html) ([2000](#_ENREF_1), [World Medical 2002](#_ENREF_17), [World Medical Association General 2004](#_ENREF_18), [World Medical Association 2009](#_ENREF_19)).

## Patients

Inclusion criteria comprised: all adult patients 18 years or older diagnosed with acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML), MDS, and CML (all phases except first CP), who received a MAC allogeneic HCT in the UK at Hammersmith Hospital, Imperial College Healthcare NHS Trust, between February 1993 to April 2011. Exclusion criteria: patients with incomplete baseline CRP data and those with CML in first CP: we previously reported analysis of this subgroup ([Pavlu*, et al* 2010](#_ENREF_12)).

Transplantation procedure

MAC consisted of two doses of cyclophosphamide 60 mg/kg daily and total body irradiation (13.2 Gy in 6 fractions). In vivo T-cell depletion was achieved with alemtuzumab for unrelated donor transplants. Prophylaxis of acute GvHD was achieved with four doses of methotrexate 8 mg/m2 and titrated cyclosporin (200-300 ng/ml) that was monitored twice weekly. Patients received supportive care, including antimicrobial prophylaxis with antibacterial, antifungal, and antiviral agents.

## CRP measurement

Preconditioning serum CRP levels were routinely measured as part of patient care at a median of 11 days (range 7–30) before transplantation using a latex immunoassay (CRP Vario®; Abbott Architect ci8200 analyzer). The normal reference range used at our institution of 0 to 9 mg/L is based on the manufacturer’s recommendation. We used this pre-defined cut-off to separate patients into two groups, CRP 0−9 mg/l and >9 mg/l ([Pavlu*, et al* 2010](#_ENREF_12)).

## HCT-CI, EMBT risk categories, covariates

The EBMT score combines five variables: disease stage, recipient age, donor type, donor/recipient sex combination, and interval from diagnosis to transplantation ([Gratwohl*, et al* 2009](#_ENREF_7)). These individual EBMT score components as well as the aggregated overall score were used in analyses ([Aki*, et al* 2012](#_ENREF_2), [Gratwohl*, et al* 1998](#_ENREF_6), [Pavlu*, et al* 2010](#_ENREF_12)). Disease stage was assessed in accordance with EBMT criteria ([Gratwohl*, et al* 2009](#_ENREF_7)): early: untreated MDS, acute myeloid leukaemia (AML)/acute lymphoblastic leukaemia (ALL)/MDS in first complete remission (CR1); intermediate: MDS/AML/ALL in second complete remission (CR2) or partial response (PR), CML accelerated phase (AP), CP after AP; late: MDS/AML/ALL in >CR2/second partial response/CML blast phase (BP).

Comorbidities were defined and assigned weights (1 to 3) according to the HCT-CI ([Sorror, et al 2005](#_ENREF_15)). HCT-CI groups of 0 to 2 were combined and compared to the group of ≥3 ([Aki*, et al* 2012](#_ENREF_2), [Artz*, et al* 2008](#_ENREF_3), [ElSawy*, et al* 2015](#_ENREF_4), [Sorror*, et al* 2015](#_ENREF_14)). The EBMT scores of 0-3 were grouped and compared to the group of ≥4 ([Aki*, et al* 2012](#_ENREF_2), [Pavlu*, et al* 2010](#_ENREF_12), [Rezvani*, et al* 2012](#_ENREF_13)). Data on cytomegalovirus (CMV) IgG serology were included, as they have been shown to be of prognostic significance ([Gratwohl*, et al* 2009](#_ENREF_7), [Ljungman*, et al* 2011](#_ENREF_10)). Data relating to transplantation decade were also included to account for improvements in supportive care.

## Data extraction

Survival outcome data and causes of death were cross-referenced between hospital records and the NHS Spine Services Portal database, part of the Health and Social Care Information Centre. Data extraction was blinded to CRP group. Data on pre-transplant comorbidities were extracted by structured review of medical records and cross-referenced with a departmental electronic database by medically qualified investigators that were blinded to CRP group, survival, and other transplant outcomes. Patient records were updated from these sources and with information from written notifications, where applicable.

## Statistical analyses

Analyses was conducted in accordance with EMBT guidelines ([Iacobelli and Committee 2013](#_ENREF_9)). Probability curves were calculated using the Kaplan-Meier method for survival and the cumulative incidence procedure for NRM, relapse and chronic GvHD. Groups were compared using the log rank test or Gray’s test ([Gray 1988](#_ENREF_8)) as appropriate, whilst Cox regression and Fine and Gray models ([Fine and Gray 1999](#_ENREF_5)) were constructed for multivariate analyses. Covariates were included in multivariate models if *P* ≤ 0.10. The chi-squared test was used to compare acute GvHD groups. In order to examine covariate factors in good risk patients, subgroup analyses were performed: in patients with low risk HCT-CI values 0-2 ([Aki*, et al* 2012](#_ENREF_2), [Artz*, et al* 2008](#_ENREF_3), [ElSawy*, et al* 2015](#_ENREF_4), [Sorror*, et al* 2015](#_ENREF_14)) and or EBMT score of 0-3 ([Aki*, et al* 2012](#_ENREF_2)). Two sided tests were used and *P* ≤ 0.05 was considered statistically significant. SPSS version 22 (IBM, New York, USA) and R version 3.1.3 (The R Foundation for Statistical Computing) for Windows were used for analyses.

# Results, additional details

## Patient cohort characteristics

Patients were classified into disease stage based on EBMT definitions (Table S1). Patients transplanted for AML (n = 34) or ALL (n = 36) in CR1 and MDS either untreated or in CR1 (n = 1) were classified as early stage (n = 71). Those with CML in AP (n = 61), in second (n = 34) or third (n = 6) CP, AML (n = 13) or ALL (n = 3) in CR2, and MDS in CR2 or PR (n = 20) were classified as intermediate stage (n = 137). Patients with CML in BP (n = 16) and acute leukaemia in >CR2 or in relapse (ALL, n = 7 and AML, n = 22) were considered late stage (n = 45).

HCT was peripheral blood in 95 (38%) patients, and bone marrow in 158 (62%) patients. The haematopoietic cell donor was an HLA-identical sibling for 120 (47%) recipients. One hundred and thirty three patients received progenitor cells from an HLA-matched (10/10) unrelated donor.

## Univariate analyses

### Validation of the HCT-CI and the aggregated EBMT score, along with its components

We validated the HCT-CI using the pre-defined cut-off of 0-2 and >2 for NRM at 100 days, 15.9% (95% CI 11–22) versus 35.6% (95% CI 25–50; *P* < 0.001), and survival at five years, 41.3% (95% CI 34–47) versus 33.6% (95% CI 21–48; *P* = 0.047) (Fig 2A and C). We also validated the aggregated EBMT score with the pre-defined cut-off of ≤3 and >3 in our cohort for NRM 14.6% (95% CI 10–23) versus 25.0% (95% CI 19–3428; *P* = 0.0375) and survival at five years 47.7% (95% CI 37–58) versus 30.9 (26–41; *P* = 0.007). The only additional covariate forming part of the EBMT score associated with NRM and survival was intermediate or advanced disease stage (*P* = 0.006 and *P* <0.001, respectively). Older age (*P* < 0.001) and the presence CMV IgG seropositivity (*P* = 0.007) were associated with an increase in NRM but not survival, whereas transplantation from 2002 onwards was associated with improved survival (*P* = 0.01) but not NRM. The other EBMT score components of donor cell source, gender combination, and disease duration prior to transplant were not significantly associated with NRM or survival (Table S2).

### CRP is associated with inferior early and long-term survival

We split the cohort in to two pre-defined groups: normal (0–9 mg/l) and elevated CRP (>9 mg/l). Patients with an elevated CRP (N = 80) experienced a NRM at 100 days of 33.7% (95% CI 25–46) and survival at five years of 27.3% (95% CI 19–39), compared to 13.0% (95% CI 9–19) and 47.6% (95% CI 40–56) for patients with a normal CRP (N = 173), respectively (Table S2). Therefore, compared to patients with a normal CRP, those with an elevated CRP experienced excess NRM of 22.2% (*P* < 0.001) and reduced survival at five years of 20.3% (*P* < 0.001) (Fig 2B and D).

### Increasing CRP level is associated with inferior early and long-term survival

To determine whether an increased CRP level is associated with an increased NRM and reduced survival, we divided the normal and elevated CRP groups into four groups in total: CRP <5mg/l, 5–9 mg/l, >9–50 mg/l and >50 mg/l. Compared to the CRP <5 mg/l group, the NRM hazard ratio (HR) for the CRP group of 5–9 mg/l was 3.1 (95% CI 1.3–7.1; P = 0.009), for the >9–50 mg/l group the HR increased to 4.1 (95% CI 1.9–9.2; P = 0.001), and for the group of >50 mg/l the HR increased further to 7.1 (95% CI 3.1–16.2; P < 0.001). There was also a similar increasing relationship between these CRP groups and the HR for survival: Compared to the CRP <5 mg/l group, the survival HR for the CRP group of 5–9 mg/l was 1.36 (95% CI 0.9–2.1; P = 0.18), for the >9–50 mg/l group the HR increased to 1.99 (95% CI 1.3–3.0; P = 0.001), and for the group of >50 mg/l the HR increased further to 2.7 (95% CI 1.6–4.3; P < 0.001). Thus, increasing CRP levels are associated with increased NRM and reduced survival.

# References

(2000) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *JAMA,* **284,** 3043-3045.

Aki, S.Z., Suyani, E., Bildaci, Y., Cakar, M.K., Baysal, N.A. & Sucak, G.T. (2012) Prognostic role of pre-transplantation serum C-reactive protein levels in patients with acute leukemia undergoing myeloablative allogeneic stem cell transplantation. *Clin Transplant,* **26,** E513-521.

Artz, A.S., Wickrema, A., Dinner, S., Godley, L.A., Kocherginsky, M., Odenike, O., Rich, E.S., Stock, W., Ulaszek, J., Larson, R.A. & van Besien, K. (2008) Pretreatment C-reactive protein is a predictor for outcomes after reduced-intensity allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant,* **14,** 1209-1216.

ElSawy, M., Storer, B.E., Pulsipher, M.A., Maziarz, R.T., Bhatia, S., Maris, M.B., Syrjala, K.L., Martin, P.J., Maloney, D.G., Sandmaier, B.M., Storb, R. & Sorror, M.L. (2015) Multi-centre validation of the prognostic value of the haematopoietic cell transplantation- specific comorbidity index among recipient of allogeneic haematopoietic cell transplantation. *Br J Haematol,* **170,** 574-583.

Fine, J.P. & Gray, R.J. (1999) A proportional hazards model for the subdistribution of a competing risk. *Journal of the American Statistical Association,* **94,** 496-509.

Gratwohl, A., Hermans, J., Goldman, J.M., Arcese, W., Carreras, E., Devergie, A., Frassoni, F., Gahrton, G., Kolb, H.J., Niederwieser, D., Ruutu, T., Vernant, J.P., de Witte, T. & Apperley, J. (1998) Risk assessment for patients with chronic myeloid leukaemia before allogeneic blood or marrow transplantation. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation. *Lancet,* **352,** 1087-1092.

Gratwohl, A., Stern, M., Brand, R., Apperley, J., Baldomero, H., de Witte, T., Dini, G., Rocha, V., Passweg, J., Sureda, A., Tichelli, A., Niederwieser, D., European Group for, B., Marrow, T. & the European Leukemia, N. (2009) Risk score for outcome after allogeneic hematopoietic stem cell transplantation: a retrospective analysis. *Cancer,* **115,** 4715-4726.

Gray, R.J. (1988) A Class of K-Sample Tests for Comparing the Cumulative Incidence of a Competing Risk. *Annals of Statistics,* **16,** 1141-1154.

Iacobelli, S. & Committee, E.S. (2013) Suggestions on the use of statistical methodologies in studies of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant,* **48 Suppl 1,** S1-37.

Ljungman, P., Hakki, M. & Boeckh, M. (2011) Cytomegalovirus in hematopoietic stem cell transplant recipients. *Hematol Oncol Clin North Am,* **25,** 151-169.

Moher, D., Hopewell, S., Schulz, K.F., Montori, V., Gotzsche, P.C., Devereaux, P.J., Elbourne, D., Egger, M. & Altman, D.G. (2010) CONSORT 2010 explanation and elaboration: updated guidelines for reporting parallel group randomised trials. *BMJ,* **340,** c869.

Pavlu, J., Kew, A.K., Taylor-Roberts, B., Auner, H.W., Marin, D., Olavarria, E., Kanfer, E.J., MacDonald, D.H., Milojkovic, D., Rahemtulla, A., Rezvani, K., Goldman, J.M., Apperley, J.F. & Szydlo, R.M. (2010) Optimizing patient selection for myeloablative allogeneic hematopoietic cell transplantation in chronic myeloid leukemia in chronic phase. *Blood,* **115,** 4018-4020.

Rezvani, K., Kanfer, E.J., Marin, D., Gabriel, I., Rahemtulla, A., Taylor, A., Macdonald, D., Dazzi, F., Milojkovic, D., Foroni, L., Pavlu, J., Sargent, J., Le Dieu, R., Goldman, J.M., Apperley, J. & Szydlo, R. (2012) EBMT risk score predicts outcome of allogeneic hematopoietic stem cell transplantation in patients who have failed a previous transplantation procedure. *Biol Blood Marrow Transplant,* **18,** 235-240.

Sorror, M.L., Logan, B.R., Zhu, X., Rizzo, J.D., Cooke, K.R., McCarthy, P.L., Ho, V.T., Horowitz, M.M. & Pasquini, M.C. (2015) Prospective Validation of the Predictive Power of the Hematopoietic Cell Transplantation Comorbidity Index: A Center for International Blood and Marrow Transplant Research Study. *Biol Blood Marrow Transplant,* **21,** 1479-1487.

Sorror, M.L., Maris, M.B., Storb, R., Baron, F., Sandmaier, B.M., Maloney, D.G. & Storer, B. (2005) Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood,* **106,** 2912-2919.

von Elm, E., Altman, D.G., Egger, M., Pocock, S.J., Gotzsche, P.C. & Vandenbroucke, J.P. (2007) The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet,* **370,** 1453-1457.

World Medical, A. (2002) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *J Postgrad Med,* **48,** 206-208.

World Medical Association General, A. (2004) World Medical Association Declaration of Helsinki: ethical principles for medical research involving human subjects. *J Int Bioethique,* **15,** 124-129.

World Medical Association, I. (2009) Declaration of Helsinki. Ethical principles for medical research involving human subjects. *J Indian Med Assoc,* **107,** 403-405.

# Tables

Table S1. Pre-myeloablative allogeneic haematopoietic cell transplant patient characteristics. Patients transplanted in first chronic phase of CML were not included as this cohort has been previously reported ([Pavlu*, et al* 2010](#_ENREF_12)). Abbreviations: CMV (cytomegalovirus); CML (chronic myeloid leukaemia); AML (acute myeloid leukaemia; ALL (acute lymphoblastic leukaemia); myelodysplastic syndromes (MDS); European Group for Blood and Marrow Transplantation (EBMT); haematopoietic cell transplantation comorbidity index (HCT-CI); number (N). The number of patients with missing data are outlined.

|  |  |
| --- | --- |
|  | N (%)  |
| Median age - years (range) | 37 (17 – 63) |
| Haematopoietic cell source  HLA matched (10/10) family donor HLA matched (10/10) unrelated donor | 120 (47%)133 (53%) |
| Disease CML (all except first chronic phase) AML ALL MDS | 117 (46.2%)69 (27.3%)46 (18.2%)21 (8.3%) |
| EBMT disease stage Early Intermediate Late | 71 (28.1%)137 (54.2%)45 (17.8%) |
| Patient CMV serology status Negative Positive *Missing* | 103 (40.7%)145 (57.3%)*5 (2%)* |
| HCT-CI score 0 1 2 ≥3*Missing* | 79 (31.2%)72 (28.5%)32 (12.6%)59 (23.3 %)*11 (4.3%)* |

Table S2: Univariate analyses of covariates associated with the primary outcomes of non-relapse mortality (NRM) at 100 days and survival at five years post transplant. Probabilities 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).

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | Non-relapse mortality (NRM) at 100 days | Overall survival at five years |
| Covariate  | **N** | **Probability (%)** **(95% CI)** | **p-value** | **Probability (%) (95%CI)** | **p-value** |
| Overall | **253** | **19.8 (16-25)** | **-** | **41.9 (35-47)** | **-** |
| CRP level 0-9 mg/l >9 mg/l | 17380 | 13.0 (9-19)33.7 (25-46) | <0.001 | 47.6 (40-56)27.3 (19-39) | <0.001 |
| HCT-CI 0-2 ≥3 *Missing* | 18359*11* | 15.9 (11-22)35.6 (25-30) | <0.001 | 41.3 (34-47)33.6 (21-48) | 0.047 |
| EBMT Score 0-3 ≥4 *Missing* | 124128*1* |  14.6(10-23)25.0 (19-34) | 0.037 | 47.7 (37-58)30.9 (26-41) | 0.007 |
| Recipient age <20 years ≥20-40 years >40 years | 1314298 | 014.9 (10-22) 29.6 (22-40) | 0.004 | 61.5 (40-95)38.4 (31-48)36.4 (27-48) | 0.25 |
| EBMT disease stage Early Intermediate Late | 7113745 | 12.8 (7-24)18.2 (13-26)35.6 (24-53) | 0.007 | 51.5 (39-67)39.3 (32-44)16.8 (8-35) | <0.001 |
| Duration of disease prior to transplantation <1 year ≥1 year | 150103 | 20.8 (16-32)17.3 (12-25) | 0.31 | 41.9 (34-52)34.5 (26-45) | 0.23 |
| Donor source matching Identical sibling Unrelated | 120133 | 22.7 (16-32)17.3 (12-25) | 0.30 | 45.8 (37-57)33.0 (25-43) | 0.11 |
| Recipient (R)/donor (D) gender combination Other combination Male (R) / Female (D) *Missing* | 19557*1* |  20.7 (16-27)16.5 (9-31)  | 0.63 | 39.8 (33-48)37.3 (25-53) | 0.70 |
| Recipient CMV IgG serology Not detected Detected *Missing* | 103145*5* | 11.8 (7-20)25.2 (19-34) | 0.006 | 35.3 (27-47)41.6 (34-52) | 0.74 |
| Transplant decade 1993-2001 2002-2011 | 132121 | 23.5 (17-32)15.5 (10-24) | 0.11 | 31.8 (25-41)47.9 (39-59) | 0.01 |

Table S3: Causes of early and late mortality stratified by preconditioning C-reactive protein levels (0–9 mg/l and >9 mg/l). Abbreviations: C-reactive protein (CRP); graft versus host disease (GvHD), number (N).

|  |  |  |
| --- | --- | --- |
|  | Within the first 100 days post HCT | Within five years post HCT |
|  | CRP level | TotalN (%) | CRP level | TotalN (%) |
| Cause of death | 0–9 mg/lN (%) | >9 mg/lN (%) | 0–9 mg/lN (%) | >9 mg/lN (%) |
| Severe sepsis or septic shock | 12 (55) | 17 (59) | 29 (57) | 32 (36) | 25 (44) | 57 (39) |
| GvHD | 7 (32) | 5 (17) | 12 (24) | 19 (21) | 10 (18) | 29 (20) |
| Disease relapse or progression | 0 (0) | 2 (7) | 2 (4) | 29 (32) | 15 (26) | 44 (30) |
| Other | 3 (14) | 5(17) | 8 (16) | 10 (11) | 7 (12) | 17 (12) |
| Total | 22 (100) | 29 (100) | 51 (100) | 90 (100) | 57 (100) | 147 (100) |

# Figures

Figure S1. CONSORT flow diagram of patients in this cohort study, grouped by normal (0-9 mg/l) and elevated (>9 mg/l) C-reactive protein (CRP).

Included (n=253)

## Enrolment

Assessed for eligibility (n= 264)

### Primary outcome:

### survival

## Follow-up

Excluded (n= 11)

  Incomplete CRP data (n=11)

## Allocation

Overall survival (n=80)

Lost to follow-up (n= 1)

Elevated CRP: >9 mg/l (n=80)

Overall survival (n=173)

Lost to follow-up (n=10)

Normal CRP: 0–9 mg/l (n=173)