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Effect of Red-Cell Transfusion Events on Transplant Related Mortality and Overall Survival in Children with Leukemia Undergoing Hematopoietic Stem Cell Transplant

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Master of Science
Clinical Research

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Abstract

Effect of Red-Cell Transfusion Events on Transplant Related Mortality and Overall Survival in Children with Leukemia Undergoing Hematopoietic Stem Cell Transplant

By Jennifer Andrews

Recent clinical studies in adult oncology patients have shown that an elevated ferritin level prior to hematopoietic stem cell transplant (HSCT), serving as a surrogate marker of body iron load, is independently associated with transplant related mortality (TRM) and inferior overall survival (OS) in adult oncology patients. There is emerging evidence in the pediatric leukemia population that the same may be true. We hypothesized that another marker of body iron load, high red-blood cell (RBC) transfusion events, may be associated with TRM and inferior OS in a heterogeneous group of children with leukemia treated with HSCT.

We performed a retrospective cohort study of 112 children with leukemia treated at our institution over the last 10 years. Approximately 1/3 of the children had high RBC event number, which we defined as greater than 12 RBC transfusion events prior to HSCT, and 2/3 had low RBC event number. Both groups were similar in regards to age, diagnoses, donor type (matched related, matched unrelated, mismatched related, mismatched unrelated), stem cell source (peripheral blood, umbilical cord, bone marrow), baseline liver, cardiac and renal function, and median follow-up time. However, more children in the low RBC transfusion event cohort had high performance scores (83.5% vs 54.5%, $p = 0.001$) and fewer had recurrent leukemia or other forms of advanced disease compared with the children more heavily transfused (41.8% vs 87.9%, $p < 0.0001$).

We found no association between number of transfusion events and TRM in our patients in both univariate and multivariate analysis. Patients with a high RBC event number did have an inferior 5-year OS (39%, 95% confidence interval [CI] 22 – 55%) compared with patients with low RBC events (56%, 95% CI 42 – 68%). However, when controlling for patient age, stage of disease, type of transplant, and performance score, number of RBC events lost prognostic significance. Likely more advanced disease and lower performance score in the high RBC event cohort was responsible for the survival difference.

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Introduction:

Iron is a physiologically essential element for many biochemical processes in the body (6). There is an elegant system of iron uptake, storage and transport; however the body has no passive excretory mechanism should overload occur. Iron is sequestered by proteins in the body (transferrin in the circulation and ferritin within cells) such that in the normal state, there is not an appreciable amount of free iron in the plasma or tissues. With exogenous load of iron, such as with chronic red-blood cell (RBC) transfusions which contain a considerable amount of iron, the body's sophisticated iron balance is upset such that free iron accumulates. Free iron causes organ damage through the production of reactive oxygen species and their interaction with organic molecules in tissues (9).

Iron overload from chronic transfusion therapy causes extensive organ damage (16). Patients with β-thalassemia require chronic RBC transfusions to sustain life, and the resulting iron overload causes considerable cardiac, hepatic and endocrine damage. In those patients who undergo hematopoietic stem cell transplant (HSCT) for cure, extent of organ damage is adversely associated with survival (12).

Recent studies have suggested that transfusional iron overload may be adversely predictive on HSCT outcomes for adult patients with malignancies as well, even in the absence of known organ damage (1, 2, 3, and 14). The only published study in pediatrics also suggests that iron overload prior to HSCT is

independently predictive of transplant related mortality (TRM) and inferior overall survival (OS) in a homogeneous group of Korean children with hematologic malignancies (11).

We performed a retrospective cohort study of a heterogeneous group of pediatric patients with hematologic malignancies who were treated with HSCT at our institution during the last decade to investigate the association between iron load, as measured by number of RBC transfusions, and TRM and OS.

Background:

Iron uptake, transport and storage in the body are controlled by elegant mechanisms such that there is never appreciable free iron in the blood circulation or cells. Red-blood cells (RBCs) contain hemoglobin, which in turn contains iron. Chronic RBC transfusions overwhelm the body's balancing mechanism such that free iron can accumulate and cause organ damage through the generation of reactive oxygen species (10).

In patients with β-thalassemia, who require chronic RBC transfusions to sustain life, iron overload is an independent prognostic risk factor for HSCT survival (12). Recent research shows that transfusional iron overload is an independent risk factor for inferior HSCT outcomes in patients with oncologic diagnoses as well.

Evidence in adult oncology patients:

Pullarkat and researchers at City of Hope National Medical Center prospectively studied 190 adult patients with acute leukemias, myelodysplasia, chronic myelogenous leukemia (CML), myeloproliferative disease and aplastic anemia undergoing matched sibling or matched unrelated donor HSCT. Ferritin, a widely accepted marker of tissue iron overload, was used in this study to estimate body iron load. Patients with elevated pretransplant ferritin, defined as

≥ 1000 nanogram/milliliter (ng/mL), had increased TRM at day 100 (20% versus 9%, p = 0.038) and decreased OS (25% versus 65%, p = 0.004) compared with patients without elevated pretransplant ferritin in univariate analysis. In multivariate analysis, controlling for diagnosis category, donor type, preparative conditioning strength, disease activity and gender, high ferritin versus ferritin < 1000 ng/mL was associated with increased TRM at day 100 (odds ratio [OR] = 3.82, p = 0.013) and decreased OS (hazard ratio [HR] = 2.28, p = 0.004) (14).

Armand and researchers at Dana-Farber Cancer Institute retrospectively studied 590 adult patients with various cancers who underwent myeloablative allogeneic HSCT at their institution for whom a pretransplantation ferritin was available. Similarly, they found an elevated pretransplant serum ferritin (in the top quartile, ≥ 2640 ng/mL) versus low was independently associated with significantly inferior OS in those patients specifically with acute leukemia (HR = 1.6, p = 0.031), when controlling for age, stage of leukemia, cytogenetic risk group, graft source, degree of match, preparative conditioning regimen, graft versus host disease (GVHD) prophylaxis, cytomegalovirus (CMV) seropositivity of recipient and donor and history of prior HSCT (3).

Interestingly, they also included pretransplantation serum albumin in their analysis to account for the role of ferritin as an acute phase reactant in the body. Albumin acts as a negative acute phase reactant among its' many functions, and the authors postulated that if the association between ferritin and mortality was dependent on acute phase reactivity (such as occurs with acute

infection), the inclusion of albumin in their model would diminish the prognostic impact of ferritin. With inclusion of albumin in their analyses, the impact of ferritin on outcome was unchanged and thus felt to be due to iron overload rather than acute phase reactivity (3).

Evidence in pediatric oncology patients:

Lee and researchers at Seoul National University College of Medicine retrospectively reviewed 101 pediatric patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), juvenile myelomonocytic leukemia (JMML), aplastic anemia and neuroblastoma who underwent HSCT at their institution. In univariate analysis, high pretransplant serum ferritin (>1000 ng/mL) was associated with worse TRM ($p = 0.0008$) compared with low serum ferritin. This adverse relationship remained significant in multivariate analysis when controlling for age, sex, diagnosis, conditioning regimen, time from diagnosis to HSCT, type of transplant and history of earlier HSCT ($p = 0.033$). In addition, unadjusted OS was worse in the high ferritin group (40% versus 80%) compared with the low ferritin group ($p = 0.001$) (11).

Moreover, 43 children with ferritin >1000 ng/mL were treated with an iron-chelating agent prior to HSCT per standard of care at their institution. The mean decrease in serum ferritin in these children was 1298 ng/mL. In these children, TRM and OS were not statistically different from those children with serum ferritin < 1000 ng/mL prior to HSCT. These authors suggest that more

intensive use of iron-chelating agents, even in children without organ damage, would improve OS and TRM related to iron overload prior to HSCT (11).

Methods:

Null Hypothesis:

TRM in children treated with HSCT for leukemia with high RBC transfusion events was equivalent to TRM in children treated with HSCT for leukemia with low RBC transfusion events.

OS in children treated with HSCT for leukemia with high RBC transfusion events was equivalent to OS in children treated with HSCT for leukemia with low RBC transfusion events.

Specific Aims:

The primary aim was to compare TRM in children with leukemia with high red-cell transfusion events prior to HSCT to TRM in children with leukemia with low red-cell transfusion events prior to HSCT.

A secondary aim was to compare OS in children with leukemia with high red-cell transfusion events prior to HSCT to OS in children with leukemia with low red-cell transfusion events prior to HSCT.

Study Design:

This study was a retrospective cohort study of pediatric patients with leukemia treated with HSCT at Children's Healthcare of Atlanta at Egleston between January 2000 and January 2010. Institutional review board approval was granted prior to data collection by Emory University and Children's Healthcare of Atlanta.

Patient Selection:

Inclusion criteria:

1. Patients between the ages of one and 21 years with a diagnosis of ALL, AML, CML, chronic myelomonocytic leukemia (CMML), JMML or acute bilineage/biphenotypic leukemia who underwent HSCT between January 2000 and January 2010 at Children's Healthcare of Atlanta at Egleston
2. Patients were eligible regardless of relapse status or remission status (induction failure, partial remission, complete remission one or greater), type of donor (matched sibling donor, partially matched related donor, matched unrelated donor, partially matched unrelated donor), type of hematopoietic stem cell source (bone marrow, umbilical cord blood, peripheral blood) or type of conditioning regimen prior to HSCT

Exclusion criteria:

1. Patients treated for their leukemia at an outside institution prior to HSCT (since transfusion records were unavailable)
2. Pregnant or breastfeeding patients per standard of care

Outcome and Predictor variables:

The primary outcome variable was TRM, measured as death prior to 180 days after HSCT from any cause other than relapse. OS was also analyzed. Predictor variables included: patient age, race, gender, diagnosis, stage of disease (induction failure, partial remission, complete remission one or greater), source of hematopoetic stem cells (bone marrow, umbilical cord blood, peripheral

blood), conditioning regimen prior to HSCT (reduced intensity or full strength), donor relatedness (matched sibling donor, partially matched related donor, matched unrelated donor, partially matched unrelated donor), Karnofsky or Lansky performance status score, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, glomerular filtration rate (GFR), shortening fraction (SF) and ejection fraction (EF) on echocardiogram, donor and recipient CMV status, pulmonary function tests including forced expiratory volume (FEV1) and forced vital capacity (FVC) if patient was able to perform testing accurately, and red-cell transfusion events prior to HSCT.

Definitions:

Red-cell transfusion event number was defined as number of packed RBC transfusions given prior to HSCT. Packed RBC transfusions given on the same day were considered one event. High red-cell transfusion event number was defined as greater than 12 packed RBC transfusions, and low red-cell transfusion event number was defined as less than or equal to 12 packed RBC transfusions prior to HSCT.

Relapse consisted of leukemia relapse, whereas TRM was death resulting from any cause other than relapse by 180 days post HSCT. For OS, death from any cause was considered an event.

Early disease was defined as patients with ALL or AML in first complete remission, CML in first chronic phase or CMML. Advanced disease was defined as ALL or AML in second or greater complete remission, CML in accelerated

phase or blast phase or second or greater chronic phase, primary induction failure of ALL or AML, and JMML.

Measurement of body iron:

The gold standard measurement of body iron load is currently liver iron concentration (LIC) since the liver is the major site of iron storage in iron overload (13). However, obtaining this measurement conventionally requires a surgical biopsy under general anesthesia with its' inherent risks, as well as risks of bleeding and infection from the procedure. Researchers and clinicians commonly use serum ferritin as a surrogate marker of iron load for its convenience and wide availability. Ferritin, however, can be elevated for other reasons (i.e. infection) and thus has poor specificity for iron load (10).

Rose and colleagues prospectively studied 65 adult oncology patients to correlate blood units transfused and LIC as measured by magnetic resonance imaging (MRI) without biopsy, an accurate method to quantify iron load in the body (5). All patients were screened and tested negative for hemachromatosis, a disease marked by iron overload independent of RBC transfusions. They found a significant correlation between number of RBC units transfused and LIC ($r = 0.84$) (15).

We chose to use red-cell transfusion events as a surrogate marker of iron load because of its' significant correlation with LIC as well as its convenience of measurement. One mL of packed RBC's contains 1 milligram (mg) of iron, such that the average RBC unit in the United States contains approximately 250 mg of

free iron. Unlike ferritin, red-cell transfusion events are not affected by inflammation in the body and do not serve as a marker of acute phase reactivity.

We chose to separate high and low RBC transfusion groups based on historical data mainly in thalassemia patients showing an association between adverse clinical outcomes and serial transfusions of approximately 100 mL of RBCs/kilogram (kg) of body weight (16). In pediatric patients, clinicians transfuse RBCs in 10-15mL/kg aliquots such that 12 RBC transfusions total approximately 120mL of RBCs/kg. There is emerging evidence specifically in pediatric ALL patients that 120mL of RBCs/kg may indeed cause iron-related organ damage (7).

Sample size:

Based on an estimated TRM rate of approximately 10% in pediatric oncology patients, to demonstrate a hazards ratio (HR) of ≥ 1.5 with 80% power and 95% confidence ($\alpha = 0.05$), a minimum sample size of 94 patients was required.

One-hundred and forty two patients met criteria for review from the Bone Marrow Transplant Database at Children's Healthcare of Atlanta. Thirty patients were excluded because treatment for their leukemia took place at an outside institution prior to HSCT, leaving 112 patients for analysis.

Statistical Analysis:

Baseline characteristics of all study participants were collected, including patient's age, race , gender, diagnosis, stage of disease (induction failure, partial remission, complete remission one or greater), source of hematopoetic stem cells (bone marrow, umbilical cord blood, peripheral blood), conditioning regimen prior to HSCT (reduced intensity or full strength), donor relatedness (matched sibling donor, partially matched related donor, matched unrelated donor, partially matched unrelated donor), Karnofsky or Lansky performance status score, total bilirubin, AST, ALT, albumin, GFR, SF or EF on echocardiogram, donor and recipient CMV status, pulmonary function tests if patient was able to perform testing accurately, and RBC transfusion events prior to HSCT. High and low RBC event groups were assessed for differences in baseline characteristics using the two sample t-test for continuous variables and χ^2 test or Fisher's exact test for categorical variables.

TRM for high and low RBC event groups were estimated using cumulative incidence curves taking into account the competing risk of relapse, and compared with the Gray's test (8). OS for each group was estimated from the time of HSCT using Kaplan-Meier curves, and compared with the log-rank statistic.

A multivariate analysis was then performed to control for the imbalance of baseline covariates in the two groups. TRM for each group was estimated using a Cox proportional hazards model taking into account competing risk (relapse) controlling for age (older or >13 years, versus younger or ≤ 13 years of age), stage of disease (advanced versus early), type of donor (alternative donor versus

matched related donor) and Karnofsky/Lansky performance status score (≥ 90 versus < 90). The results of this model correspond to the cause-specific hazard instead of the regular marginal hazard. The cause-specific hazard was defined as the instantaneous failure rate at time t from a specific type of transplant-related mortality given that the patient was still alive prior to time t (neither death nor death from relapse has occurred) (8). OS for each group was estimated using a Cox proportional hazard model controlling for age (older versus younger), stage of disease (advanced versus early), type of donor (alternative donor versus matched related donor) and Karnofsky/Lansky performance status score (≥ 90 versus < 90).

Univariate analysis and Cox proportional hazard modeling for OS was performed using SAS software, version 9.2 (SAS Institute, Inc., Cary, NC, USA). Cumulative incidence estimates and Cox proportional hazard modeling for TRM accounting for competing risk was performed using R software, version 2.12.0, 'cmprsk' package. A cutoff level of $p \leq 0.05$ (two-sided) was used for assessing statistical significance.

Results:

Baseline Characteristics:

Baseline characteristics of the high and low RBC exposure groups are shown in Tables 1 and 2. Thirty three patients, or approximately one-third, had high RBC event number, and 79 had low RBC event number.

Median age in both groups was similar at about 10 years of age, and approximately one-half of patients in each cohort were males. The most common diagnosis in each group was ALL (57.6% in the high RBC exposure group and 41.8% in the low RBC exposure group), with no significant differences in diagnoses between the two groups. Approximately one-half of patients in each group were Caucasian, with no significant differences in racial composition between exposure groups. There were no significant differences in donor type (matched sibling donor, partially matched related donor, matched unrelated donor, partially matched unrelated donor) or type of hematopoietic stem cell source (bone marrow, umbilical cord blood, peripheral blood) with the majority of patients in both groups receiving bone marrow as their graft source. Over 90% of children in each group had full intensity conditioning chemotherapy treatment prior to their HSCT. CMV status of recipient and donor were not significantly different between RBC exposure groups. In addition, liver function as measured by albumin, AST, ALT and bilirubin was not significantly different between the groups. Cardiac function as measured by EF and SF on echocardiogram was not significantly different between cohorts. Median follow-

up time for survivors was not significantly different between the two groups with 48 months in the high RBC group and 32.7 months in the low RBC group.

However, the majority of patients (83.5%) in the low RBC event cohort had a Karnofsky/Lansky performance status score of ≥ 90 compared with only 54.5% in the high RBC event cohort ($p = 0.001$). Disease status also differed significantly between the groups with a significantly higher proportion of children in the high RBC group having advanced stage disease (87.9%) than the children in the low RBC group (41.8%) ($p < 0.0001$). In addition, pulmonary function as measured by FEV1 and FVC (in children old enough to perform this sophisticated clinical test) and kidney function as measured by GFR were statistically different between the high and low RBC exposure groups. However, the mean values for FEV1, FVC and GFR for children in both groups were still within normal range and this statistical difference was not clinically relevant.

Transplant Related Mortality:

Cumulative incidence curves for each group are shown in Figure 1. TRM at 180 days for children exposed to high RBC event number approached 22%, whereas TRM at 180 days for children with low RBC event number approached 15%. This difference in TRM incidence was not statistically significant between cohorts ($p = 0.45$).

In multivariate analysis, we did not observe an association between RBC transfusion exposure and TRM (HR of exposure to high versus low RBC transfusion events = 0.8, 95% CI 0.3 – 2.1) (see table 3). Donor type was the only

variable associated with TRM (HR of alternative donor versus matched related donor = 5.7, 95% CI 1.3 – 25.6) when controlling for age (older versus younger), stage of disease (advanced versus early), Karnofsky/Lansky performance status score (≥ 90 versus < 90), and exposure to RBC events (high versus low).

Overall Survival:

5-year OS curves are shown in Figure 2. Children exposed to high RBC transfusion events had a 5-year OS approaching 40%, while children with low RBC events had 5-year OS approaching 60%. This survival difference was significant in univariate analysis (p = 0.0384).

However, when controlling for age (older versus younger), stage of disease (advanced versus early), type of donor (alternative donor versus matched related donor) and Karnofsky/Lansky performance status score (≥ 90 versus < 90), children exposed to high RBC transfusion events versus low RBC events were not less likely to survive (HR = 1.3, 95% CI 0.7 – 2.5) (see table 4). In addition, the hazard ratios for older versus younger, advanced disease versus early disease or for alternative donor versus matched related donor were not significant. However, the HR for Karnofsky/Lansky status score ≥ 90 versus < 90 was significant at 0.5 (95% CI 0.3 – 0.9) when controlling for the above mentioned co-variates.

Discussion:

Unlike in previously published studies in adults and children finding an association between iron overload and adverse clinical outcomes following HSCT, we did not find an association between high RBC transfusion events and TRM at 180 days post HSCT or worse OS in children with leukemia in our single-institution retrospective cohort study. Although we did find that children with high RBC transfusion events had worse 5-year OS (39%, 95% CI 22 – 55%) compared with children with low RBC events (56%, 95% CI 42 – 68%) in univariate analysis, this association lost significance in multivariate analysis when taking into account patient age, stage of disease, type of donor, and Karnofsky/Lansky performance status score. Likely this association was attributable to other patient characteristics in the high RBC transfusion event group.

Donor type was the only variable associated with TRM in our multivariate analysis (HR of alternative donor versus matched related donor = 5.7, 95% CI 1.3 – 25.6), consistent with previous studies showing that patients who receive matched related donor transplants have better survival than patients who receive alternative donor transplants (17). Karnofsky/Lansky performance status score ≥ 90 versus < 90 was the only variable significantly associated with OS in our multivariate analysis (HR = 0.5, 95% CI 0.3 – 0.9), consistent with the knowledge that poor performance status prior to HSCT is associated with TRM and thereby worse OS.

The biological explanations for our results are unclear. Children utilize iron in the body differently than adults in that iron is used for growth (4). Perhaps this utilization of free iron for growth by our young patients' accounts for the fact that high RBC transfusion events were not associated with worse outcomes.

Certainly our study is limited in that our measurement, RBC transfusions, is only a surrogate marker of total iron in the body. We do believe, however, that our surrogate measurement of iron load is more accurate than ferritin, a surrogate marker affected by inflammation and used by other authors to date investigating iron load and HSCT outcomes. We do not have data in our patients regarding true iron deposition in the organs (which could be more accurately measured with LIC via MRI, for example). We also dichotomized high and low RBC events based on data from thalassemia patients, who receive RBC transfusions typically over years. We inferred that children with leukemia should be similarly dichotomized, but this supposition may be inaccurate and may in fact result in misclassification of our exposure groups.

In addition, RBC transfusions are given in a discrete period of time in children with leukemia, usually over months rather than years. Perhaps RBC transfusions given in this way result in less iron deposition than in those patients who receive chronic transfusions over years. This biological information has not yet been well studied.

Thalassemia patients also typically have more severe anemia than children with leukemia who typically are transfused after mild to moderate anemia is

diagnosed. Iron absorption from the gastrointestinal tract increases in states of severe anemia. In individuals with severe anemia, iron absorption can be as much as two grams of iron load per year (13). Certainly this supplemental iron absorption contributes to the iron load of those children with more severe anemia than that of our typical leukemia patients.

Our study is, to date, the only investigation in a heterogeneous cohort of American children with leukemia looking at the association between iron overload and worse HSCT outcomes. It is limited because we studied children at a single institution. We plan on analyzing outcomes in children treated at other large pediatric transplant centers in a retrospective manner to see if our results are reproducible and representative of children in the U.S. It would also be valuable to study HSCT outcomes in a prospective manner in a cohort of children with leukemia using a more direct measure of iron load in the body, such as LIC measured by MRI.

In conclusion, we did not find an association between high RBC transfusion events and TRM at 180 days post HSCT or worse OS following HSCT in children with leukemia treated at our institution over the last decade. However, the role of iron deposition following RBC transfusions in children with cancer deserves further study because there are therapies available to bind free iron in the body. It's critical to better understand the pathophysiology of iron deposition following RBC transfusions in oncology patients so that clinicians can intervene with chelation therapies if necessary to improve HSCT outcomes.

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Table 1. Baseline characteristics of patients with high RBC exposure versus low RBC exposure.

Characteristic	Patients with high RBC exposure (n = 33)	Patients with low RBC exposure (n = 79)	P-value[1]
Median age (years) (range)	10.1 (1.4, 18.1)	10.2 (0.3, 18.9)	0.9
Gender (male %)	17 (51.5)	43 (54.4)	0.8
Race (%)			1.0
Caucasian	19 (57.6)	39 (49.3)	
African American	9 (27.3)	26 (32.9)	
Hispanic	4 (12.1)	9 (11.4)	
Asian	1 (3.0)	2 (2.5)	
Other	0 (0)	3 (3.8)	
Disease (%)			0.3
AML	12 (36.4)	28 (35.4)	
ALL	19 (57.6)	33 (41.8)	
Bilineage/biphenotypic	1 (3)	7 (8.9)	
CML	0 (0)	7 (8.9)	
CMML	0 (0)	1 (1.3)	
JMML	1 (3)	3 (3.8)	
Donor type (%)			0.1
Auto	1 (3)	1 (1.3)	
Matched related	8 (24.2)	37 (46.8)	
Mismatched related	5 (15.2)	8 (10.1)	
Matched unrelated	6 (18.2)	14 (17.7)	
Mismatched unrelated	13 (39.4)	19 (24.1)	
Stem cell source (%)			0.4
Peripheral blood	2 (6.1)	3 (3.8)	
Bone marrow	19 (57.5)	57 (72.2)	
Cord	10 (30.3)	15 (19)	
Double cord	2 (6.1)	4 (5)	
Performance score (%)			0.001
<90	15 (45.5)	13 (16.5)	
≥ 90	18 (54.5)	66 (83.5)	
Disease status (%)			<0.0001
Early	4 (12.1)	46 (58.2)	
Advanced	29 (87.9)	33 (41.8)	
Conditioning regimen (%)			0.2
Reduced intensity	3 (9.1)	2 (2.5)	
Full intensity	30 (90.9)	77 (97.5)	

CMV status Recipient +/Donor – Recipient +/Donor + Recipient -/Donor + Recipient -/Donor -	 11 (33.4) 14 (42.4) 4 (12.1) 4 (12.1)	 32 (40.5) 25 (31.7) 8 (10.1) 14 (17.7)	0.7
Median follow-up of survivors (months) (range)	48 (13.7, 100.8)	32.7 (4.7, 83.4)	0.1

Abbreviations: RBC red-blood cell, AML acute myeloid leukemia, ALL acute lymphoblastic leukemia, CML chronic myelogenous leukemia, CMML chronic myelomonocytic leukemia, JMML juvenile myelomonocytic leukemia, CMV cytomegalovirus.

[1] P-value calculated by two sample t-test for continuous variables and χ^2 test or Fisher's exact test for categorical variables, 2-sided p-value calculated at alpha = 0.05.

Table 2. Baseline organ function of patients with high RBC exposure versus low RBC exposure.

Measure (mean)	Patients evaluated	Patients with high RBC exposure (n = 33)	Patients with low RBC exposure (n = 79)	P-value[1]
Albumin	112	3.8	3.9	0.2
ALT	112	31.5	37.8	0.3
AST	112	33.0	38.0	0.3
Bilirubin	112	0.4	0.4	0.9
Ejection fraction	112	63.4	64.4	0.6
FEV1	72[2]	83.9	95.1	0.04
FVC	72[2]	84.5	95.9	0.04
GFR	109[3]	101.1	112.4	0.03
Shortening fraction	95[4]	34.2	35.0	0.5

Abbreviations: RBC red-blood cell, ALT alanine aminotransferase, AST aspartate aminotransferase, FEV1 forced expiratory volume, FVC forced vital capacity, GFR glomerular filtration rate.

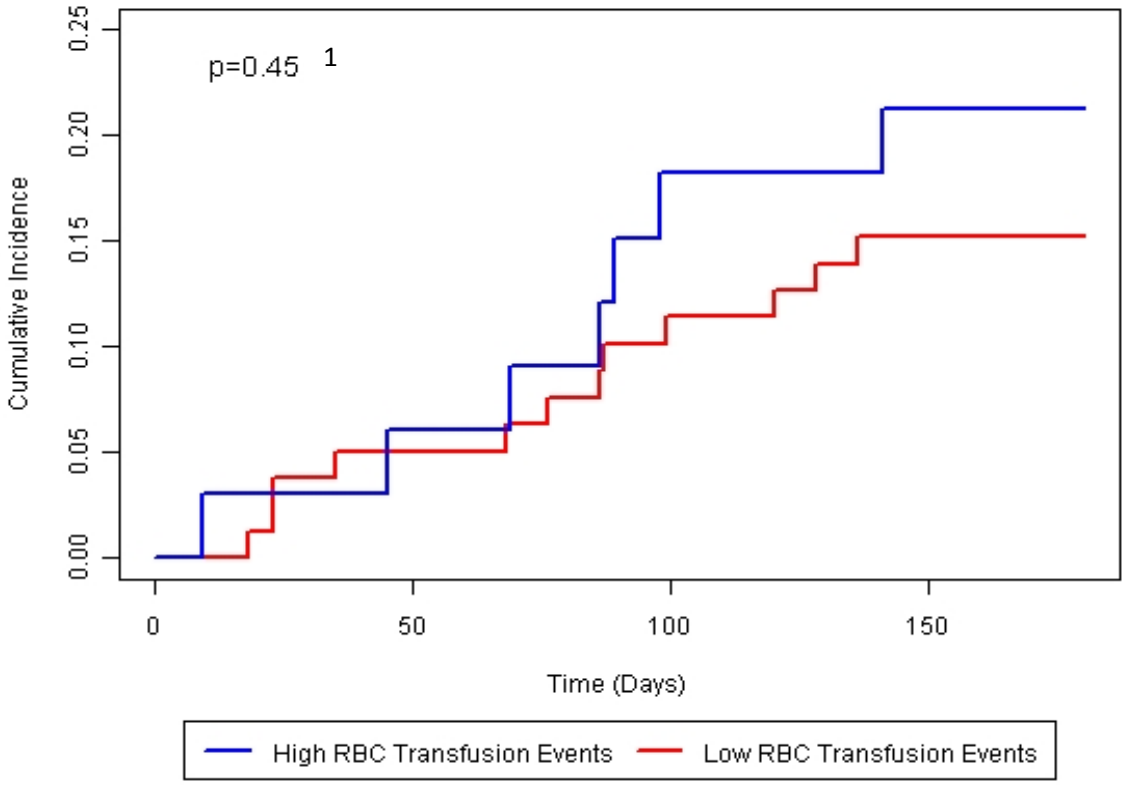
[1] P-value calculated by two sample t-test for continuous variables and χ^2 test or Fisher's exact test for categorical variables, 2-sided p-value calculated at alpha = 0.05.

[2] Some patients unable to perform pulmonary function testing due to young age.

[3] GFR not performed in 3 patients.

[4] Shortening fraction not available in some patients.

Figure 1. Cumulative incidence curves of TRM for high RBC exposure versus low RBC exposure groups.



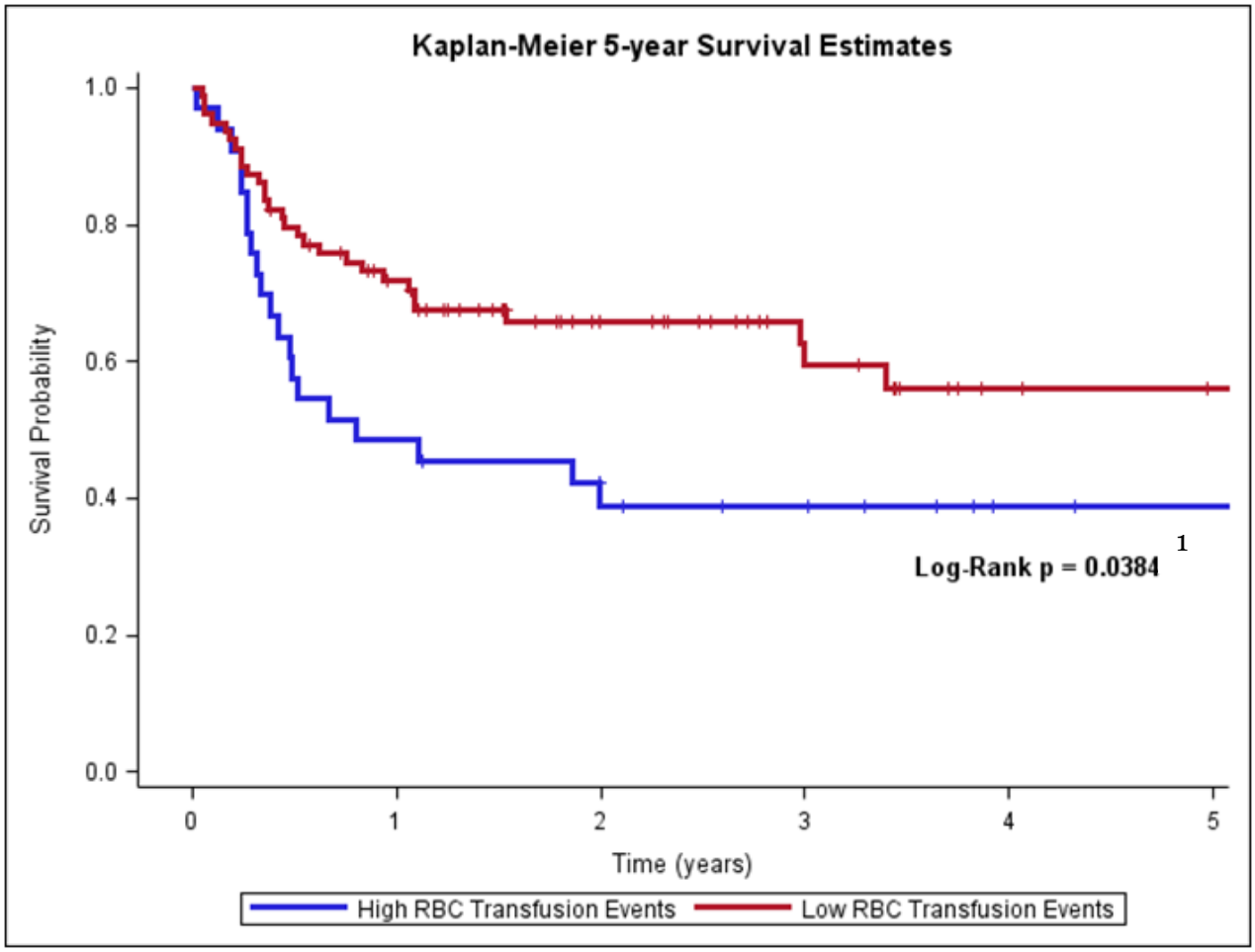
Abbreviations: TRM transplant related mortality, RBC red-blood cell.
[1] P-value calculated by Gray's test taking into account competing risk, 2-sided p-value calculated at alpha = 0.05.

Table 3. Multivariate analysis for TRM.

Characteristic	Hazard ratio	95% Confidence Interval	P-value[1]
Older age versus younger	1.1	0.4 – 2.8	0.8
Advanced stage disease versus early	1.6	0.5 – 4.9	0.4
Performance score ≥ 90 versus < 90	0.5	0.2 – 1.3	0.1
High RBC events versus low	0.8	0.3 – 2.1	0.6
Alternative donor versus matched related donor	5.7	1.3 – 25.6	0.02

Abbreviations: TRM transplant related mortality, RBC red-blood cell.
[1] P-value calculated by Cox proportional hazards model taking into account competing risk, 2-sided p-value calculated at alpha = 0.05.

Figure 2. Comparison of 5-year OS curves for high RBC exposure and low RBC exposure groups.



Abbreviations: OS overall survival, RBC red-blood cell.
[1] P-value calculated by log-rank test, 2-sided p-value calculated at alpha = 0.05.

Table 4. Multivariate analysis for OS.

Characteristic	Hazard ratio	95% Confidence Interval	P-value[1]
Older age versus younger	1.5	0.8 – 2.6	0.2
Advanced stage disease versus early	1.4	0.8 – 2.7	0.3
Performance score ≥ 90 versus < 90	0.5	0.3 – 0.9	0.02
High RBC events versus low	1.3	0.7 – 2.5	0.4
Alternative donor versus matched related donor	1.1	0.9 – 1.4	0.3

Abbreviations: OS overall survival, RBC red-blood cell.
[1] P-value calculated by Cox proportional hazards model, 2-sided p-value calculated at alpha = 0.05.