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## Observational Study

**Lymphocyte recovery is an independent predictor of relapse in allogeneic hematopoietic cell transplantation recipients for acute leukemia**

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**Abstract****AIM**

To examine the optimal absolute lymphocyte count (ALC) cut-off utilizing receiver operator characteristics (ROC) in addition to graft characteristics associated with early ALC recovery.

**METHODS**

Patients who received T-cell replete peripheral hematopoietic cell transplantation (HCT) for acute leukemia were identified. ALC cut-off was established using ROC analysis and subsequently the cohort was stratified. Time to endpoint analysis and cox regression modelling was computed to analyze outcomes.

**RESULTS**

A total of 72 patients met the inclusion criteria and

were analyzed. Optimal ALC cut-off was established to be on day 14 (D14) with  $ALC > 0.3 \times 10^9/L$ . At 2 years, cumulative incidence of relapse was 16.9% *vs* 46.9% ( $P = 0.025$ ) for early and delayed lymphocyte recovery cohorts, respectively. Chronic graft *vs* host disease was more prevalent in the early lymphocyte recovery (ELR) group at 70% *vs* 27%, respectively ( $P = 0.0006$ ). On multivariable analysis for relapse, ELR retained its prognostic significance with  $HR = 0.27$  (0.05-0.94,  $P = 0.038$ ).

### CONCLUSION

ELR is an independent predictor for relapse in patients receiving allogeneic HCT for acute leukemia. ELR was influenced by graft characteristics particularly CD34 count.

**Key words:** Acute leukemia; Allogeneic transplant; Absolute lymphocyte count

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**Core tip:** Disease relapse remains the most common cause of treatment failure after allogeneic hematopoietic stem cell transplantation for acute leukemia. Previous studies have identified that early lymphocyte recovery can be a surrogate of graft *vs* leukemia effect hence identifying high risk patients for relapse. However, published reports are heterogeneous with regards to timeline and magnitude of lymphocyte recovery. Using receiver operator characteristics with area under the curve, we identified that absolute lymphocyte count  $> 0.3 \times 10^9/L$  at day 14 is associated with half the relapse risk which was statistically significant at the multivariable analysis. There was a trend towards improved progression free survival and overall survival for patients with early lymphocyte recovery. In conclusion, we observed that lymphocyte recovery is an independent predictor of relapse in allogeneic transplant recipients for acute leukemia. This would help identify high risk patients who may benefit from maintenance strategies post-transplant.

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### INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HCT) is widely used to cure a number of hematologic malignancies including acute myeloid leukemia (AML) and lymphoblastic leukemia/lymphoma (ALL)<sup>[1-4]</sup>. Relapse of the primary disease remains the most frequent cause of treatment failure in contemporary

HCT recipients<sup>[5]</sup>. Several factors are associated with relapse such as status at HCT, associated cytogenetic abnormalities, conditioning regimen and occurrence of chronic graft *vs* host disease<sup>[6]</sup>. Prognosis after overt relapse post-HCT is very poor and a minority of patients are able to achieve durable remissions<sup>[7]</sup>. Hence, identification of patients at risk of relapse may permit preemptive interventions for relapse prevention<sup>[8]</sup>.

Immune reconstitution post HCT, particularly lymphocyte recovery, can be a surrogate for graft *vs* leukemia (GVL) effect hence improved long term disease control. Several groups reported that early absolute lymphocyte count (ALC) recovery is associated with decreased relapse rates in hematologic malignancies. However, there is heterogeneity regarding the predictive optimal threshold and timing of lymphocyte recovery. For example Michelis *et al*<sup>[9]</sup> reported that  $ALC \geq 0.5 \times 10^9/L$  on day 28 in AML patients is associated with reduction of the relapse risk at multivariable analysis with hazard ratio (HR) = 0.49 (0.26-0.92,  $P = 0.03$ ) without a survival advantage. On the other hand, Kumar *et al*<sup>[10,11]</sup> showed that  $ALC \geq 0.15 \times 10^9/L$  on day +30 resulted in a 3 fold reduction in relapse risk in AML patients but an ALC of  $> 0.17 \times 10^9/L$  on day +21 was protective from relapse in ALL patients. Thoma *et al.* showed that  $ALC > 0.3 \times 10^9/L$  on day +100 is associated with improved overall survival (OS)<sup>[12]</sup>.

In light of the above discrepancies, we examined the impact of ALC recovery on post HCT outcomes; where optimal ALC threshold and timeline was analyzed using receiver operator characteristics (ROC) and area under the curve (AUC). We also analyzed infused allograft cellular content for factors predicting early ALC recovery.

### MATERIALS AND METHODS

#### Patient selection

After due institutional review board (IRB) approval, patients  $\geq 14$  years of age with AML or ALL who underwent HCT at our institution between 2010 - 2015 were identified.

The selection criteria included patients receiving myeloablative (MAC) or reduced intensity conditioning (RIC) from related or unrelated donors. Classification of the conditioning intensity was based on the criteria suggested by the Centre of International Blood and Marrow Transplant Research (CIBMTR)<sup>[13]</sup>. Selection of regimen intensity was at the discretion of the treating physician and generally patients with a hematopoietic stem cell co-morbidity index (HCT-CI)  $< 3$  were considered for MAC regimen<sup>[14]</sup>. Patients with ALL who were candidates for MAC, preferentially received a total body irradiation (TBI) based regimen. Exclusion criteria were for patients who received a bone marrow graft or cord blood stem cell source, second transplant and those who underwent *in vivo* or *in vitro* T-cell depletion. Data were collected retrospectively from the patient's electronic medical records. Cytogenetic data

at the time of diagnosis was collected and stratified as previously described for AML patients<sup>[15]</sup>. ALL patients with hypodiploid karyotype, translocations at (4;11), (11q23), (9;22) and (1;19) were deemed high risk, and remaining patients were classified as standard risk<sup>[16-20]</sup>.

### **Preparative regimens and GVHD prophylaxis**

Patients candidates for MAC intensity received one of two regimens based on the underlying diagnosis; patients with ALL received cyclophosphamide 60 mg/kg intravenously (IV) for two days followed by 1200 cGy of TBI fractionated twice daily for three days. Patients with AML received fludarabine 30 mg/m<sup>2</sup> daily for five days in addition to busulfan 3.2 mg/kg IV daily for four days in addition to cyclophosphamide 60 mg/kg IV daily for 2 d. Mesna was given for bladder protection. For RIC regimens, patients received either fludarabine 30 mg/m<sup>2</sup> IV daily for 5 d with busulfan 3.2 mg/kg IV daily for two days or fludarabine 30 mg/m<sup>2</sup> IV daily for 5 d with melphalan 70 mg/m<sup>2</sup> IV for two days. Phenytoin loading and maintenance was given for seizure prophylaxis if busulfan was used until 24 h post last dose. Graft vs host disease (GVHD) prophylaxis consisted of methotrexate and cyclosporine. Methotrexate was given at 15 mg/m<sup>2</sup> on day +1 followed by 10 mg/m<sup>2</sup> on days +3, +6 and +11 with leucovorin rescue 24 h post each methotrexate dose. Day +11 was omitted if there is evidence of significant liver toxicity or grade  $\geq$  2 mucositis.

### **Definitions and transplant related outcomes**

OS was calculated from the date of transplant until the date of death of any cause or last documented follow-up date. Progression free survival (PFS) was calculated from the time of transplant until death of any cause or relapse. Cumulative incidence of relapse (CIR) was calculated from the date of transplant until relapse or date of last follow up. Cumulative incidence of non relapse mortality (NRM) was calculated from the date of transplant until death of any cause without evidence of disease relapse. Acute and chronic GVHD was diagnosed according to standard criteria. Neutrophil engraftment was defined as an absolute neutrophil count (ANC) of  $0.5 \times 10^9/L$  or higher for 3 consecutive days. Platelet engraftment was defined as platelet count higher than  $20 \times 10^9/L$  for 7 consecutive days without transfusion support.

### **End points**

The primary end point was to examine the impact of early ALC recovery (ELR) on CIR. Secondary endpoints were to examine effect of ELR on other post HCT outcomes (OS, PFS and NRM) and to examine infused allo-graft cellular content for factors predicting ELR. ALC was abstracted on days +7, +14, +21 and +28 from the Complete Blood Count (CBC) post HCT using either the automated or manual differential method<sup>[21]</sup>.

### **Statistical analysis**

Baseline patient, disease and treatment related variables

were reported using descriptive statistics (counts, medians and percentages). Categorical and continuous variables were compared using Pearson's  $\chi^2$  and Wilcoxon/Kruskal-Wallis, respectively. Probability of OS was computed using the Kaplan-Meier method. Group comparisons were made using the log-rank test. Time to event was calculated from the date of transplant until the event of interest or point of last clinical encounter, in which case the event will be censored. Cumulative incidence was computed as competing events using Grey's model, considering death as a competing event for relapse and relapse as a competing event for NRM. Univariable and multivariable analyses were performed using Cox proportional hazard regression modelling and expressed as HR with 95%CI and *P* value. Any variable with a *P*  $\leq$  0.1 was incorporated into the multivariable model in a stepwise selection process. Thresholds of ALC recovery post HCT as well as infused allograft characteristics, if present, were assessed using the ROC and AUC for the end point of relapse. Statistical analysis were performed using JMP Pro Version 11 (SAS Institute, Cary, NC, United States) software and EZR on R commander version 1.28<sup>[22]</sup>.

## **RESULTS**

### **Patient and transplant characteristics**

A total of 72 patients met the inclusion criteria and their data were analysed. Baseline characteristics of the cohort are shown in Table 1. Majority of transplants were from related donors (88%), while the remaining minority (12%) were from unrelated donors. Transplants were from peripheral blood stem cells, while cord blood and bone marrow grafts were excluded due to different immune reconstitution kinetics. All patients were from the Middle East and North Africa Region. The median follow up was 17 mo (range: 2-64.8) at which point the CIR was 35.2% and OS was 67.3%.

### **Optimal ALC threshold**

ROC curves with AUC were used to determine the best cut-off value for ALC on days +7, +14, +21 and +28 based on their utility as a marker for the binary outcome of relapse vs no relapse. ALC on day +14  $> 0.3 \times 10^9/L$  was identified as the optimal cut-off point. Patients were subsequently stratified as ELR if ALC on day +14  $> 0.3 \times 10^9/L$  and delayed lymphocyte recovery (DLR) if day +14 ALC was  $\leq 0.3 \times 10^9/L$ . Patient's disease and HCT related variables are stratified per lymphocyte recovery as shown in Table 1. Cohorts were similar with regards to age, gender, diagnosis, performance status, cytogenetic risk, status at HCT, stem cell source, donor gender, ABO matching and conditioning intensity. Regimens containing TBI were more common in the DLR group at 63% vs 33% (*P* = 0.019).

### **Infused allo-graft characteristics influencing ELR**

We examined infused allo-graft cellular contents for factors predicting ELR in our patients. Optimal thresholds

**Table 1** Baseline characteristics of patients stratified by lymphocyte recovery *n* (%)

Variable	ALC > 0.3 ( <i>n</i> = 24)	ALC ≤ 0.3 ( <i>n</i> = 48)	<i>P</i> value
Patient age in years, median (range)	28 (16-57)	23 (14-63)	0.57
Recipient gender, male	13 (54)	28 (58)	0.74
Diagnosis			0.54
AML	13 (54)	22 (45)	
ALL	11 (46)	26 (54)	
ECOG	1 (0-2)	0 (0-3)	0.86
Cytogenetics (AML)			0.5
Favorable	3 (25)	2 (10)	
Intermediate	7 (58)	15 (71)	
High risk	2 (17)	4 (67)	
Cytogenetics (ALL)			0.78
Standard	5 (56)	11 (50)	
High risk	4 (44)	11 (50)	
Female donor/male recipient	4 (17)	11 (23)	0.53
Related donor	21 (88)	42 (88)	1
Status at HCT			0.33
CR1	13 (54)	31 (66)	
≥ CR2	11 (46)	16 (34)	
ABO Matching			0.89
Match	16 (67)	31 (64)	
Major/bidirectional	3 (12)	8 (17)	
Minor	5 (21)	9 (19)	
TBI containing regimen	8 (33)	30 (63)	0.019
Conditioning intensity			0.19
MAC	18 (75)	42 (88)	
RIC	6 (25)	6 (12)	

HCT: Hematopoietic stem cell transplant; AML: Acute myeloid leukemia; ALL: Acute lymphoblastic leukemia; ALC: Absolute lymphocyte count; ECOG: Eastern cooperative oncology group; TBI: Total body irradiation; CR: Complete remission; MAC: Myeloablative conditioning; RIC: Reduced intensity conditioning.

were again determined by ROC with AUC analysis. We observed that infusing grafts with the following characteristics was associated with higher incidence of ELR; CD 34 of <math>6 \times 10^6/\text{kg}</math> (71% vs 42%,  $P = 0.018$ ), CD3 >  $24 \times 10^7/\text{kg}$  (19% vs 2%,  $P = 0.017$ ), infused ALC >  $1.3 \times 10^8/\text{kg}$  (96% vs 74%,  $P = 0.015$ ), infused lymphocyte-monocyte ratio (LMR) > 4 (33% vs 11%,  $P = 0.022$ ) and CD 34 <math>6 \times 10^6/\text{kg}</math> with ALC >  $1.3 \times 10^8/\text{kg}$  (67% vs 27%,  $P = 0.0012$ ). These results are shown in Table 2.

### Impact of ELR on post HCT outcomes

Stratified by lymphocyte recovery, after 2 years of follow up, the CIR was significantly higher for the DLR vs ELR groups at 46.9% vs 16.9%, respectively ( $P = 0.025$ ). On the other hand, at 2 years, there was a non-significant difference of NRM between the two cohorts at 14.2% vs 23.3% for the DLR and ERL groups, respectively ( $P = 0.51$ ). There was a trend towards improved 2 year PFS for the ELR at 61.9% vs 40.1% ( $P = 0.09$ ), but no significant difference of OS was observed at 70.1% vs 53.9% for ELR vs DLR, respectively ( $P = 0.12$ ) (Figure 1). Median time to ANC and platelet engraftment was similar for both groups at 17 (12-29) d and 24 (21-37) for ELR

**Table 2** Graft characteristics as predictors of lymphocyte recovery *n* (%)

Graft characteristic	ALC > 0.3 ( <i>n</i> = 24)	ALC ≤ 0.3 ( <i>n</i> = 48)	<i>P</i> value
CD 34 $\times 10^6/\text{kg}$ < 6	17 (71)	20 (42)	0.018
TNC > $7 \times 10^7/\text{kg}$	5 (21)	10 (21)	1
CD 3 > $24 \times 10^7/\text{kg}$	4 (19)	1 (2)	0.017
CD 34 < $6 \times 10^6/\text{kg}$ , CD 3 > $24 \times 10^7/\text{kg}$	3 (100)	0 (0)	0.0088
MNC > $2.7 \times 10^8/\text{kg}$	20 (83)	33 (69)	0.17
ALC > $1.3 \times 10^8/\text{kg}$	23 (96)	35 (74)	0.015
AMC > $1.75 \times 10^8/\text{kg}$	3 (13)	14 (30)	0.093
ALC > $1.3 \times 10^8/\text{kg}$ , CD34 < $6 \times 10^6/\text{kg}$	16 (67)	13 (27)	0.0012
LMR > 4	8 (33)	5 (11)	0.022

ALC: Absolute lymphocyte count; TNC: Total nuclear count; MNC: Mononuclear count; AMC: Absolute monocyte count; LMR: Lymphocyte-monocyte ratio.

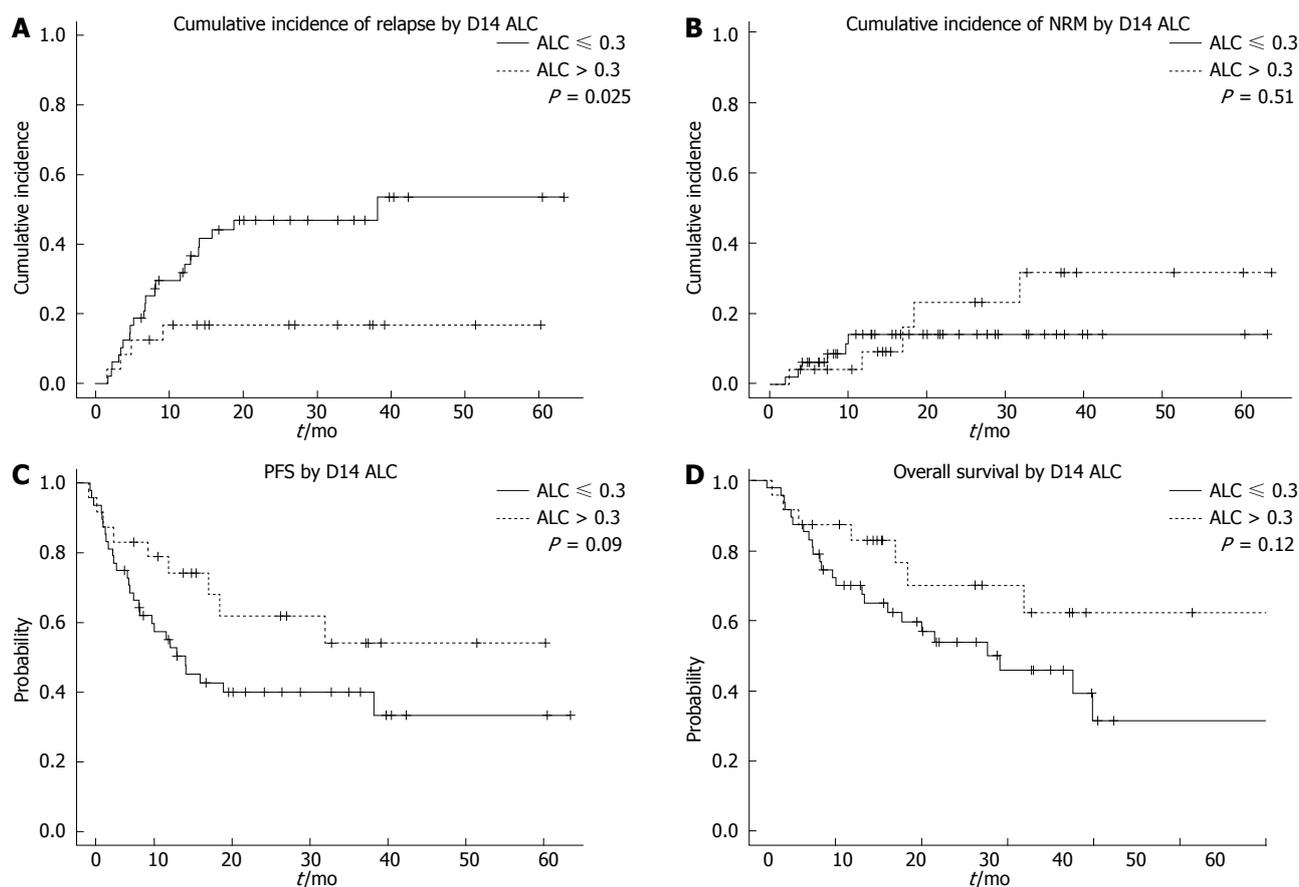
and 17 (12-25) and 24 (7-42), respectively ( $P = 0.76, 0.98$ ). Incidence of aGVHD was similar but cGVHD was significantly higher in the ELR groups at 70% vs 27% ( $P = 0.0006$ ). These results are shown in Table 3.

Six variables were found to influence relapse at univariable analysis; age at HCT HR = 0.97 (0.94-1.01,  $P = 0.1$ ), single marital status HR = 2.59 (1.13-6.65,  $P = 0.023$ ), female donor to male recipient HR = 2.15 (0.91-4.7,  $P = 0.079$ ), CR1 remission HR = 0.52 (0.23-1.15,  $P = 0.1$ ), cGVHD HR = 0.24 (0.079-0.59,  $P = 0.0013$ ) and ELR 0.31 (0.09-0.8,  $P = 0.014$ ). We also examined the impact of TBI on relapse given the higher incidence of TBI based conditioning in the DLR group, but did not see an apparent impact with HR = 1.003 (0.46-2.2,  $P = 0.99$ ). Three factors remained prognostic at the multivariable analysis which were ELR HR = 0.27 (0.05-0.94,  $P = 0.038$ ), CR1 remission HR = 0.36 (0.15-0.87,  $P = 0.024$ ) and cGVHD 0.33 (0.1-0.92,  $P = 0.035$ ). These results are shown in Table 4.

Causes of mortality in the ELR and DLR cohorts were related to relapse of primary disease in 3/8 (38%) and 18/24 (75%), infection 1/8 (12%) vs 0/24, organ failure 0/8 vs 1/24 (4.2%), aGVHD 1/8 (12) vs 2/24 (8.3%) and cGVHD 3/8 (38%) vs 3/24 (12%). These results are shown in Table 5.

## DISCUSSION

The present analysis highlights again the value of ELR as a protective factor from disease relapse in acute leukemia. In particular, we report that ALC >  $0.3 \times 10^8/\text{kg}$  on day +14 post allogeneic HCT for acute leukemia is an independent factor predicting decreased CIR at multivariable analysis. We also observed a trend towards improved PFS and OS; however this did not meet statistical significance. NRM was not significant between both cohorts, however both the incidence of cGVHD and cGVHD related deaths were more frequent in the ELR group. Incidence of cGVHD related deaths



**Figure 1** Post transplantation outcome of cumulative incidence of relapse (A), cumulative incidence of non-relapse mortality (B), progression free survival (C), and overall survival (D) stratified by lymphocyte recovery on day 14. ALC: Absolute lymphocyte count; PFS: Progression free survival.

Table 3 Transplant related outcomes			
Variables	ALC > 0.3 (n = 24)	ALC ≤ 0.3 (n = 48)	P value
CIR (2-yr)	16.90%	46.90%	0.025
NRM (2-yr)	23.20%	14.20%	0.51
PFS (2-yr)	61.90%	40.10%	0.09
OS (2-yr)	70.10%	53.90%	0.12
Plt engraftment (median, d)	24 (21-37)	24 (7-42)	0.98
ANC engraftment (median, d)	17 (12-29)	17 (12-25)	0.76
aGVHD	5 (22)	15 (31)	0.4
cGVHD	16 (70)	13 (27)	0.0006

ALC: Absolute lymphocyte count; CIR: Cumulative incidence of relapse; NRM: Non-relapse mortality; PFS: Progression free survival; OS: Overall survival; Plt: Platelet; ANC: Absolute neutrophil count; aGVHD: Acute or chronic graft vs host disease; cGVHD: Chronic graft vs host disease.

was 37.5% (3/8) in the ELR group compared to 12.5% (3/24) in the DLR group. This perhaps explains the lack of statistical significance seen for PFS and OS.

Give that graft source and manipulation can affect cellular reconstitution post-transplant, we excluded patients who received bone marrow or cord blood grafts in addition to those receiving T-cell depleted manipulation of the graft<sup>[23,24]</sup>. TBI was administered more frequently in the DLR group, but we did not observe an impact on relapse using TBI at the univariable analysis level with HR: 1 (0.46-2.2, P = 0.99).

At multivariable analysis, three factors had an impact on relapse: CR1, cGVHD and ELR. cGVHD is well described to decrease incidence of relapse due to a parallel GVL effect<sup>[25]</sup>. The current analysis supports the hypothesis that ELR is a surrogate for GVL as cGVHD incidence was significantly higher in the ELR group. Incidence of cGVHD related deaths were also more frequent in the ELR group, which likely accounts for the observed NRM, PFS and OS rates.

Although lymphocyte subsets were not identified in this analysis, the most plausible subset implicated in our analysis would likely be the natural killer (NK) cells as they represent the bulk of recovered lymphocytes by two weeks post HCT<sup>[26]</sup>. Previously, NK cells were found to be an independent factor predicting post HCT outcomes in T-cell depleted grafts<sup>[27]</sup>. However, this finding was not reproduced when T-cell replete grafts were used<sup>[28]</sup>. That said, this observed protective effect from ELR is likely a complex interplay between various lymphocyte subsets, such as NK cells, cytotoxic T-lymphocytes (CD8+) and regulatory T-cells (CD4+ and CD25+)<sup>[29,30]</sup>. Furthermore, the infused graft cellular content likely impacts post HCT reconstitution, and this has been well demonstrated in the autologous HCT setting and to a lesser extent allogeneic HCT<sup>[12,31-33]</sup>.

Infused allo-graft cellular content predicts post HCT reconstitution. We observed that higher T-cell

**Table 4 Univariable and multivariable risk factors influencing incidence of relapse**

IR	Univariable HR (95%CI, P value)	Multivariable HR (95%CI, P value)
Age at HCT	0.97 (0.94-1.01, P = 0.1)	0.13 (0.0096-1.38, P = 0.093)
Single Marital status	2.59 (1.13-6.65, P = 0.023)	0.82 (0.21-3.27, P = 0.77)
AML <i>vs</i> ALL	0.82 (0.36-1.8, P = 0.62)	
Female D <i>vs</i> Male R	2.15 (0.91-4.7, P = 0.079)	2.24 (0.88-5.31, P = 0.086)
Match <i>vs</i> Mismatch	1.9 (0.3-6.7, P = 0.42)	
MRD <i>vs</i> Other	1.6 (0.47-10, P = 0.49)	
D14 ALC > 0.3	0.31 (0.09-0.8, P = 0.014)	0.27 (0.05-0.94, P = 0.038)
MAC <i>vs</i> RIC	1.38 (0.46-3.4, P = 0.53)	
CR1 <i>vs</i> other	0.52 (0.23-1.15, P = 0.1)	0.36 (0.15-0.87, P = 0.024)
aGVHD	0.54 (0.16-1.43, P = 0.23)	
cGVHD	0.24 (0.079-0.59, P = 0.0013)	0.33 (0.1-0.92, P = 0.035)

ALC: Absolute lymphocyte count; HR: Hazard ratio; CR1: First complete remission; R: Recipient; D: Donor; AML: Acute myeloid leukemia; ALL: Acute lymphoblastic leukemia; cGVHD: Chronic graft *vs* host disease; MAC: Myeloablative conditioning; RIC: Reduced intensity conditioning; MRD: Matched related donor.

**Table 5 Causes of mortality stratified by absolute lymphocyte count recovery**

Variables	ALC > 0.3 (n = 8)	ALC ≤ 0.3 (n = 24)
Primary disease	3	18
Infection	1	N/A
Organ failure	N/A	1
aGVHD	1	2
cGVHD	3	3

ALC: Absolute lymphocyte count; aGVHD: Acute or chronic graft *vs* host disease; cGVHD: Chronic graft *vs* host disease; N/A: Not available.

and absolute lymphocyte content was significantly associated with ELR. Higher CD34 content is typically associated with faster engraftment and decreased rejection<sup>[34,35]</sup>. The National Marrow Donor Program (NMDP) reported on a cohort of over 900 unrelated HCTs using peripheral blood stem cells indicating that higher CD34 doses resulted in rapid engraftment, decreased transplant related mortality (TRM) and improved OS using various conditioning regimens<sup>[36]</sup>. However, the median stem cell dose administered was  $6 \times 10^6/\text{kg}$  and  $5 \times 10^6/\text{kg}$  in myeloablative (MAC) and reduced intensity RIC transplants, respectively. We found that infusing  $< 6 \times 10^6/\text{kg}$  stem cells was significantly associated with ELR. This is consistent with other reports indicating that administering higher doses of stem cells leads to detrimental outcomes both in MAC and RIC regimens<sup>[37-40]</sup>. Collectively, it appears that the optimal stem cell dose is  $6-8 \times 10^6/\text{kg}$ , thus striking a balance between (GVL) and GVHD<sup>[41]</sup>.

This analysis has inherent limitations, primarily due to the retrospective nature and sample size. We excluded patients who had T-cell manipulation or grafts other than peripheral blood stem cells as these factors can impact immune reconstitution. However, a number of important observations were made. First, similar to prior reports, we observed that ELR is protective of relapse but the timing post HCT and lymphocyte thresholds were determined using ROC-AUC and not empirically. Second, a higher incidence of cGVHD

and cGVHD related deaths was seen with ELR, which confirms the likely mechanism of lower CIR seen in this cohort. Interestingly, marital status was significantly associated with decreased CIR although it did not retain significance at the multivariable analysis. Lastly, we reported that infusing less stem cells correlates better with ELR thus challenging the notion of "more is better".

In summary, the presented study demonstrates an independent protective effect of ALC at 14 d post allogeneic HCT. Given that patients with acute leukemia relapsing after allogeneic HCT have a dismal prognosis. Early identification of these cases may facilitate preemptive decisions such as early cessation of immune-suppression or use of lymphocyte infusion in order to better harness the GVL effect, or other maintenance strategies such as hypomethylating agents. These important observations warrant further study.

## COMMENTS

### Background

Disease relapse remains the most common cause of treatment failure after allogeneic hematopoietic stem cell transplantation for acute leukemia. Several factors are associated with relapse such as status at hematopoietic cell transplantation (HCT), associated cytogenetic abnormalities, conditioning regimen and occurrence of chronic graft *vs* host disease. Prognosis after overt relapse post-HCT is very poor and a minority of patients are able to achieve durable remissions. Hence, identification of patients at risk of relapse may permit preemptive interventions for relapse prevention. Immune reconstitution post HCT, particularly lymphocyte recovery, can be a surrogate for GVL effect hence improved long term disease control.

### Research frontiers

Several groups reported that early absolute lymphocyte count (ALC) recovery is associated with decreased relapse rates in hematologic malignancies. However, there is heterogeneity regarding the predictive optimal threshold and timing of lymphocyte recovery.

### Innovations and breakthroughs

The authors examined the impact of ALC recovery on post HCT outcomes. Using receiver operator characteristics with area under the curve, the authors identified that absolute lymphocyte count  $> 0.3 \times 10^9/\text{L}$  at day 14 is associated with half the relapse risk which was statistically significant at the multivariable analysis. The authors also observed that infused graft content influences ALC

recovery.

### Applications

Given that patients with acute leukemia relapsing after allogeneic HCT have a dismal prognosis. Early identification of these cases may facilitate pre-emptive decisions such as early cessation of immune-suppression or use of lymphocyte infusion in order to better harness the GVL effect, or other maintenance strategies such as hypomethylating agents.

### Terminology

ALC recovery post allogeneic HCT is an easy to measure marker and can be used as a surrogate to identify high risk patients for relapse. Using receiver operator characteristics with area under the curve can help identify the optimal ALC threshold to exhibit this protective effect.

### Peer-review

This is an interesting study, demonstrating lymphocyte recovery as independent predictor for relapse in allogeneic hematopoietic stem cell transplantation for acute leukemia.

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