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## Original Article

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# Impact of CD34 Positive Cell Dose on Engraftment in Patients with Acute Leukemias receiving Allogeneic Stem Cell Transplant

## Abstract

**Objective:** To evaluate the impact of CD34+ cell dosing and transplant type on engraftment measures, complications, and survival in a resource-limited setting.

**Methodology:** A retrospective cross-sectional study was conducted on 70 patients (Matched: 61.4%; Haploidentical: 38.6%) diagnosed with acute leukemia at a tertiary care center in Pakistan (2016–2023). Variables included CD34+ dose, engraftment timing, GVHD incidence, and 5-year survival, reported as per STROBE guidelines.

**Results:** The mean CD34+ dose was  $8.8 \times 10^6$ /kg. Neutrophil engraftment was faster in matched transplants (13.7 vs. 16.1 days), though haploidentical grafts showed a higher cumulative success rate (92.6%). Pediatric patients received the highest CD34+ doses and achieved the fastest platelet recovery (9.7 days). At Day 100, successful neutrophil recovery was higher in the matched group (62.7% vs. 48.1%). Acute GVHD and graft failure were more frequent in the haploidentical cohort (37.0% and 25.9%, respectively), while chronic GVHD was higher in matched recipients (18.6%). The 5-year survival was 30.2% for matched and 14.8% for haploidentical transplants, significantly perplexed by high attrition rates (20.9% and 37% lost to follow-up).

**Conclusion:** Higher CD34+ dosing correlates with enhanced hematopoietic recovery, particularly in pediatric cohorts. While haploidentical transplants are worthwhile alternatives, they face higher rates of acute complications and attrition. Optimizing transplant outcomes in resource-limited settings requires both personalized clinical protocols and resilient longitudinal patient-tracking infrastructure.

**Keywords:** Acute Leukemia, CD34+ Cell Dose, Engraftment, GVHD, Haploidentical Transplant.

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## Introduction

Allogeneic hematopoietic stem cell transplant (HCT) has significantly improved the outcome of high-risk acute leukemia. Neutrophil engraftment typically observed between 10 to 28 days post-transplant, while platelet engraftment generally takes place between 14 to 42 days. This duration may vary depending on factors like transplant type and patient-specific characteristics (age, gender or WBC counts)<sup>1</sup>, requiring close monitoring for signs of engraftment and potential complications during this period.

CD34 serves as a marker of hematopoietic stem cells, supporting in their quantification and extraction from peripheral blood.<sup>2</sup> Leukapheresis is the method employed to isolate CD34+ cells from blood and bone marrow using targeted antibodies. Modifying the optimal

CD34+ cell dose, one should consider factors like patient age, disease severity, prior therapies, and transplant protocols to enhance engraftment and treatment efficacy.<sup>3</sup>

The dose of CD34 counts impacting allogeneic transplant varies internationally and within Asia. There is less published information, as there are variations in practice and outcomes among different countries and regions.

The CD34+ cell dose required for allogeneic HCT depends on histocompatibility levels for HLA-matched donors.<sup>4</sup> An adequate dose ranges from 2 to  $5 \times 10^6$  CD34+ cells/kg, whether from a matched sibling donor (MSD) or matched unrelated donor (MUD). In HLA-mismatched or haploidentical transplantation, a higher dosage of 10 to  $20 \times 10^6$  CD34+ cells/kg is typically administered.<sup>5,6</sup> The graft source can be taken either through peripheral blood stem cells or from bone marrow.<sup>7</sup>

Higher amounts of CD34 counts are associated with better engraftment and reduced risk of graft failure, however it can increase the risk of GVHD. This

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complexity is influenced by various factors, including transplant protocol, patient's age, underlying disease, conditioning regimen intensity and donor recipient compatibility.<sup>8,9</sup>

Prior investigations on the association between CD34 cell dose and long-term survival outcomes have shown variation. The purpose of this study is to evaluate the impact of optimal CD34+ cell dosing on engraftment measures and the incidence of Graft-versus-Host Disease (GVHD) in acute leukemia patients, highlighting how conditioning regimen and prophylaxis protocols can align outcomes in resource-limited settings with those of matched and haplo identical transplants.

To assess long-term survival and relapse incidence while identifying the influence of conditioning regimens and patient-specific variables (age, gender, and diagnosis) on transplant efficacy in a resource-limited setting.

## Methodology

A retrospective cross sectional study was conducted at Aga Khan University Hospital, Karachi, Pakistan, from 1st January 2016 till 31st December 2023. The study received approval from the Ethical Review Committee (ERC) of Aga Khan University (ERC #: 2024-9971-29837) and the College of Physicians and Surgeons of Pakistan (CPSP). The patient data was extracted using the electronic medical record numbers of the institute, ensuring patient confidentiality.

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Study variables included patients' demographic characteristics (age, gender); transplant-related factors such as donor type (matched or haploidentical transplant), CD34-positive cell dose and its impact on engraftment, immunosuppressive therapy, and GVHD prophylaxis; post-transplant complications; and clinical outcomes, including overall survival.

Sample size was calculated using the Openepi software and the non-probability consecutive sampling technique was used. To conduct statistical analysis for CD34 positive cell dose using SPSS and descriptive statistics such as means, standard deviations, frequencies, and percentages. The study reported in accordance with the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.

**Inclusion criteria:** The study included patients of all age groups who underwent allogeneic hematopoietic stem cell transplant with HLA-matched or haplo-identical donor in acute leukemia, stem cells acquired either from peripheral blood or bone marrow or both, and a myeloablative conditioning regimen along with specific protocols used for haplo-identical.

**Exclusion criteria:** Patients who underwent allogeneic stem cell transplant for benign disorders.

The primary objective of the study to evaluate the impact of CD34+ cell dosing and transplant type on engraftment measures in patients with acute leukemia undergoing allogeneic hematopoietic stem cell transplantation (allo-HSCT). The secondary objective of the study to compare clinical outcomes, including the incidence of acute and chronic Graft-versus-Host Disease (GVHD), infectious complications (CMV/BK virus), and graft failure between matched and haploidentical transplant modalities.

**Engraftment:** Defined as the first of 3 consecutive days of an absolute neutrophil count of  $\geq 0.5$ . Platelet recovery was recorded as the first of 7 consecutive days with a platelet count  $\geq 20$  K/ $\mu$ L without transfusion.

## Results

The study included 70 patients with acute leukemia, comprising acute myeloid leukemia (AML; n = 27), B-cell acute lymphoblastic leukemia (B-ALL; n = 36)—including Philadelphia chromosome-positive (n = 24) and Philadelphia chromosome-negative (n = 12) cases—and T-cell acute lymphoblastic leukemia (T-ALL; n = 6). The cohort consisted predominantly of male patients (62.3%), with females accounting for 37.7%, and a mean age of 26.3 years.

Haploidentical hematopoietic stem cell transplantation was performed in 27 patients (38.6%), while 43 patients (61.4%) underwent fully matched transplantation. Patients receiving haploidentical transplants were

younger, with a mean age of 23.3 years, compared with 28.2 years in the fully matched transplant group.

Neutrophil engraftment was achieved faster in the fully matched cohort (13.7 vs. 16.1 days); however, a higher cumulative success rate was noted in the haploidentical group (92.6% vs. 86.0%). Platelet engraftment time were similar between transplant types 13.5 vs. 11.9 days (88.9 vs. 88.4%), though pediatric patients showed the fastest recovery overall (9.7 days). Males had slightly longer engraftment times in the haplo-identical group. CD34+ cell doses were comparable across both groups (Haplo: 9.1, Full:  $8.7 \times 10^6/\text{kg}$ ). (Table )

Diagnosis influenced engraftment patterns; Ph+ B-ALL and AML cohorts demonstrated faster recovery (13.6 and 14.4 days; 92% success) compared to Ph- B-ALL and T-ALL cohorts, which showed slower kinetics (14.7 and 15.7 days) and reduced success rates (88% and 83%). The patients were classified into 3 groups based on the CD34+ cell dose received: low,  $2-5 \times 10^6$  cells/kg; moderate  $5-8 \times 10^6$  cells/kg; and high,  $>8 \times 10^6$  cells/kg. Mean CD34+ dose:  $8.8 \pm 4.0 \times 10^6/\text{kg}$  (range: 1.14-24.5). Most patients received very high CD34+ doses i.e. 60% of patients, with only a small proportion receiving low to moderate doses. Pediatric patients received highest CD34+ dosing ( $10.0 \pm 3.2 \times 10^6/\text{kg}$ ) while adolescents the lowest ( $6.8 \pm 3.0 \times 10^6/\text{kg}$ ). Minimal dose variation observed in gender based CD34+ cell dose yield.

The CY/TBI regimen was the most frequently used conditioning protocol; however, BU/CY was associated with the fastest engraftment, with a mean time of  $12.6 \pm$

2.6 days. Age-stratified analysis demonstrated no statistically significant correlation between age and engraftment time, although a stronger negative trend was observed among adolescent patients.

Regarding GVHD prophylaxis, cyclosporine plus methotrexate was the most commonly employed regimen (64.3%) and was associated with a mean engraftment time of 14.3 days and the lowest complication rate (24.4%). Alternative prophylactic strategies used primarily in haploidentical transplantation, including tacrolimus/mycophenolate mofetil and cyclosporine/mycophenolate mofetil combined with post-transplant cyclophosphamide (PTCy), demonstrated comparable engraftment times but were associated with higher complication rates (44.4% and 57.1%, respectively).

Overall, these findings indicate that while engraftment measures were similar across prophylactic regimen, cyclosporine/methotrexate was associated with a more favorable safety profile.

The complication rates demonstrated discrete patterns based on donor type. Chronic Graft-Versus-Host Disease (cGVHD) was significantly more prevalent in the fully matched cohort (n=8, 18.6%) compared to the haploidentical group (n=2, 7.4%). Conversely, the incidence of acute GVHD (aGVHD) and graft failure was higher in the haploidentical cohort, though these differences did not reach statistical significance (aGVHD: 37.0% vs. 23.2%; Graft failure: 25.9% vs. 13.9%, respectively).

**Table I: Correlation of CD34+ Cell Dose with Hematopoietic Recovery.**

| Transplant Type       | CD34+ Dose ( $\times 10^6/\text{kg}$ ) | Neutrophil Engraftment (Days) | Neutrophil Success (%) | Platelet Engraftment (Days) | Platelet Success (%) |
|-----------------------|----------------------------------------|-------------------------------|------------------------|-----------------------------|----------------------|
| Fully Matched (n=43)  | 8.7                                    | 13.7                          | 86.0%                  | 11.9                        | 88.4%                |
| Haploidentical (n=27) | 9.1                                    | 16.1                          | 92.6%                  | 13.5                        | 88.9%                |

**Table II: Comparative Analysis of Post-Transplant Complications.**

| Complication              | Fully Matched (n=43) | Haploidentical (n=27) | Overall (N=70) |
|---------------------------|----------------------|-----------------------|----------------|
| Graft-Versus-Host Disease |                      |                       |                |
| Acute GVHD                | 10 (23.2%)           | 10 (37.0%)            | 20 (28.6%)     |
| Chronic GVHD              | 8 (18.6%)            | 2 (7.4%)              | 10 (14.3%)     |
| Graft Outcomes            |                      |                       |                |
| Graft Failure             | 6 (13.9%)            | 7 (25.9%)             | 13 (18.6%)     |
| Infectious Complications  |                      |                       |                |
| CMV Reactivation          | 9 (20.9%)            | 10 (37.0%)            | 19 (27.1%)     |
| BK Virus                  | 1 (2.3%)             | 2 (7.4%)              | 3 (4.3%)       |
| Infection-Free            | 33 (76.7%)           | 15 (55.6%)            | 48 (68.6%)     |

**Table III: Long-term Survival, Relapse Incidence, and Day 100 Chimerism.**

| Outcome Measure                   | Matched Transplant (N=43) | Haploidentical Transplant (N=27) |
|-----------------------------------|---------------------------|----------------------------------|
| 8-Year Relapse Incidence          | 7 (16.2%)                 | 5 (18.5%)                        |
| Survival Status (Final Follow-up) |                           |                                  |
| Alive                             | 13 (30.2%)                | 4 (14.8%)                        |
| Deceased                          | 21 (48.8%)                | 13 (48.1%)                       |
| Lost to Follow-up                 | 9 (20.9%)                 | 10 (37.0%)                       |
| Day 100 Chimerism (>95% Donor)    |                           |                                  |
| Achieved                          | 23 (53.4%)                | 16 (59.2%)                       |
| Not Assessed / Missing            | 20 (46.6%)*               | 11 (40.8%)*                      |

Majority (68.6%) of cohort maintained an infection-free post-transplant course, however affected 31.4% of the total study population. Viral reactivations were the predominant infectious concern, with a higher burden observed in haploidentical recipients. Specifically, Cytomegalovirus (CMV) reactivation was noted in 37% of haploidentical patients compared to 20.9% of matched recipients. Similarly, BK virus incidence was higher in the haploidentical group (7.4% vs. 2.3%). (Table II)

The 8-year relapse incidence was 16.2% (n=7) in the matched transplant group and 18.5% (n=5) in the haploidentical group. Regarding long-term survival, 30.2% (n =13) in the fully matched group were alive at the end of the follow-up period, while 48.8% (n =21) deaths were recorded and 20.9% (n =9) patients were lost to follow-up. In the haploidentical cohort, 14.8% (n =4) remained alive, 48.1% (n =13) died, and 37.0% (n = 10) were lost to follow-up. Full-match transplants had slightly better survival (20.9%) compared to haplo-identical transplants (15.4%), though both had similar mortality rates. By Day 100, chimerism assessed showed >95% in 23 patients of matched transplant (53.4%) while haploidentical 16 patients (59.2%), however 44.3% (n =31) had no chimerism assessment. (Table III)

At the Day 100 landmark assessment, successful neutrophil engraftment ( $ANC > 0.5 \times 10^9/L$ ) was observed in 62.7% (n=27) of the matched transplant cohort, compared to 48.1% (n=13) in the haploidentical group. Conversely, delayed or incomplete neutrophil recovery was noted in 16.2% (n=7) and 18.5% (n=5) of the matched and haploidentical groups, respectively. Regarding megakaryocytic recovery, matched transplants demonstrated a higher rate of platelet engraftment above the clinical threshold at day 100 (41.8%, n=18) compared to the haploidentical cohort

(18.5%, n=5). Notably, a higher proportion of haploidentical patients (48.1%, n=13) remained below the platelet threshold at Day 100, highlighting a potential delay in platelet engraftment within this subgroup. (Table )

**Table IV: Comparative Engraftment Status at Day 100 Post-HSCT.**

| Parameter                                         | Matched Transplant (N=43) | Haploidentical Transplant (N=27) |
|---------------------------------------------------|---------------------------|----------------------------------|
| Neutrophil Recovery ( $ANC > 0.5 \times 10^9/L$ ) |                           |                                  |
| Above Threshold                                   | 27 (62.7%)                | 13 (48.1%)                       |
| Below Threshold                                   | 7 (16.2%)                 | 5 (18.5%)                        |
| Not Assessed / Missing                            | 9 (20.9%)                 | 9 (33.3%)                        |
| Platelet Recovery ( $> 100 \times 10^9/L$ )       |                           |                                  |
| Above Threshold                                   | 18 (41.8%)                | 5 (18.5%)                        |
| Below Threshold                                   | 16 (37.2%)                | 13 (48.1%)                       |
| Not Assessed / Missing                            | 9 (20.9%)                 | 9 (33.3%)                        |

## Discussion

The study consisting of 70 patients who underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) diagnosed with acute leukemia, provides a profound understanding of the impact of transplant types, CD34+ cell dose, conditioning regimens, and prophylactic approaches on engraftment phase, complications, and post-transplant survival outcomes. The findings highlight the variation in treatment outcomes based on patient-specific factors and underscore the appropriateness of personalized transplant strategies.

Neutrophil engraftment was slower in haplo-identical transplants compared to full-match, yet showed a higher overall success rate. This supports evidence that, with proper immunosuppression, haplo-identical transplants can be effective alternatives when matched donors are unavailable.<sup>13,14</sup> A similar pattern was seen for platelet engraftment outcomes, with slightly longer time in haplo-identical transplants compared to full-match transplants but almost identical success rates, as reported by another study.<sup>15</sup>

Engraftment outcomes were found to be remarkably influenced by age. The fastest platelet recovery was achieved by the pediatric patients; on the contrary, the slowest platelet recovery was seen in the adolescents. This could be the result of the highest CD34+ dosing in pediatric patients, which potentially favored their improved outcomes, as also shown by an earlier study.<sup>16,17</sup> In contrast, literature has shown delayed

platelet engraftment at the lowest doses, which was the case in our adolescent cohort.<sup>18</sup> Given these age-specific outcomes, CD34+ dose needs to be taken into consideration when developing treatment protocols, which should be planned differently based on the age groups to optimize transplant outcomes, as backed by a recent study finding.<sup>19</sup>

The impact of increasing age on neutrophil engraftment was inversely related in our study, even though several studies have not found any significant difference in engraftment rates by age groups.<sup>20,21</sup> Similarly, a negative correlation was observed between the dose of CD34+ cells and platelet engraftment, suggesting that more stem cell input enhances hematopoietic recovery, confirming prior reports linking higher CD34+ doses with faster platelet recovery.<sup>22,23</sup> The positive correlation between absolute neutrophil count (ANC) and platelet engraftment further supports a shared biological trajectory during hematopoietic recovery. The majority of our cohort received very high CD34+ doses and achieved a high success rate for engraftment. Gender-based dosing showed no significant differences, which could be related to the notion that males and females have similar yields of CD34+ cells.<sup>24</sup>

Engraftment in B-ALL shows more adequate results with CD34+ doses of  $>8 \times 10^6/\text{kg}$ , while T-ALL may benefit from customized conditioning regimens and enhanced GVHD prophylaxis.<sup>25,26</sup> AML patients had stable outcomes, supporting current transplant practices with recommended CD34+ doses of  $8 \times 10^6/\text{kg}$ .<sup>27</sup> The engraftment success rate continued to be high for most of these subtypes, reinforcing the significance of focusing on disease biology and modifiable patient-dependent factors for optimization of CD34+ dose after a certain threshold.<sup>28</sup>

Conditioning regimens significantly impacted engraftment and GVHD rates; although CY/TBI was more commonly used, BU/CY showed faster engraftment but both had the high GVHD incidence as consistent with previous study.<sup>29</sup> In contrast, FLU/MEL and ATG-containing or PTCY (post-transplant cyclophosphamide) regimens yielded optimal outcomes with low GVHD rate, supporting evidence that T cell depletion reduces GVHD and enhances early engraftment.<sup>30-32</sup>

While all GVHD prophylaxis strategies had similar engraftment times, Cyclosporine/Methotrexate showed

the lowest complication rates and remains the gold standard<sup>33,34</sup>, whereas PTCY/Cyclosporine/MMF demonstrates higher, comparable to that seen in a large retrospective study.<sup>35</sup> Additionally, high CMV-related infection rates, especially in full-match transplants, emphasize the need for targeted prophylaxis in immunosuppressed, CMV-positive patients.

Major complications observed in our study included acute GVHD grade II-IV and chronic GVHD, graft failure, and disease relapse. Acute GVHD and graft failure were more frequent in the haploidentical cohort (37.0% and 25.9%, respectively), while chronic GVHD was higher in matched recipients (18.6%), aligning with globally reported data<sup>36,37</sup>, followed by chronic GVHD, while graft failure and relapse occurred at similarly lower rates, indicating acceptable long-term morbidity for the cohort. Overall, these observations reinforce the essential role of preemptive measures to curb post-transplant complications and improve survival outcomes.

Our study shows a 48.6% mortality rate, reflecting the aggressive nature of acute leukemia, with In this study, the observed 5-year survival rates of 30.2% for matched and 14.8% for haploidentical transplants are significantly confounded by high attrition rates of 20.9% and 37%, respectively. This significant loss to follow-up introduces potential predisposition, limiting the reliability of long-term survival interpretations and highlighting a critical need for improved patient assessment system. Ultimately, these findings emphasize that in resource-limited settings, enhancing registry infrastructure is as vital to optimizing allo-HSCT outcomes as refining clinical dosing protocols.<sup>38</sup> Day 100 recovery rates for neutrophils and platelets were lower than early engraftment rates, and incomplete chimerism data revealed gaps in long-term monitoring, stressing the importance of stronger post-transplant follow-up systems.

Our study has limited by its retrospective design, small sample size, single-center data, and incomplete long-term follow-up, emphasizing the need for future studies with vigorous design and follow-up to better assess allogeneic HSCT outcomes in acute leukemia.

## Conclusion

The results of our study highlight the decisive role of CD34+ dosing in optimizing engraftment outcomes and

post-transplant usefulness in acute leukemia patients. Modifying transplant types and conditioning regimens based on patient-specific factors along with enhanced prophylactic strategies to limit post-transplantation complications helps in improving disease outcomes. This study represents a comprehensive regional evaluation of allo-HSCT in acute leukemia and provides clinically actionable understandings for enlightening transplant protocols.

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