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Comparative Analysis of Corrected Total Nucleated Cells (TNC) versus CD34 Counts and Their Impact on Graft Outcomes in Allogeneic Bone Marrow Transplant: A Study in a Resource-Limited Setting

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Abstract

Objective: To compare corrected total nucleated cell (TNC)-based and CD34-selected grafts regarding engraftment, graft-versus-host disease (GVHD), and survival outcomes in allogeneic hematopoietic stem cell transplantation in a resource-limited setting.

Methodology: This retrospective study included 84 patients who underwent allogeneic hematopoietic stem cell transplantation at Gambat Institute of Medical Sciences between January 2019 and February 2024. Patients were divided into CD34-selected (n=44) and corrected TNC-based (n=40) graft groups. Demographic and clinical characteristics, graft parameters, neutrophil and platelet engraftment, acute and chronic GVHD, graft failure, overall survival (OS), and disease-free survival (DFS) were compared between groups. Statistical analysis was performed using SPSS version 26.0, with $p<0.05$ considered statistically significant.

Results: The mean age was 35.2 years in the CD34 group and 36.1 years in the TNC group (p=0.68). Graft characteristics and infusion volumes were comparable. Successful engraftment occurred in 86.4% and 80.0% of patients in the CD34 and TNC groups, respectively (p=0.42), with median engraftment times of 16 and 17 days (p=0.55). Acute GVHD occurred in 22.7% versus 35.0% and chronic GVHD in 18.2% versus 25.0% of patients, respectively (p=0.42). Primary graft failure occurred in 9.1% and 15.0% (p=0.42). One-year OS was 79.5% versus 70.0% (p=0.31), while three-year OS was 68.2% versus 60.0% (p=0.40). DFS outcomes were also comparable.

Conclusion: Corrected TNC-based grafts demonstrated outcomes comparable to CD34-selected grafts in terms of engraftment, GVHD, and survival. Corrected TNC quantification may provide a practical and cost-effective alternative in resource-limited transplant centers where CD34 selection facilities are unavailable.

Keywords: Allogeneic Bone Marrow Transplant, CD34 + Cell Counts, Total Nucleated Cells (TNC), Graft Outcomes in Resource Limited Settings.

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Introduction

Bone marrow transplantation (BMT) is recognized as the first-line therapy for many hematological malignant and nonmalignant diseases.[1] This process includes application of Hematopoietic stem cells (HSCs) which are collected from the compatible donor of the patient.[2] This is alongside administration of a preparative regimen which is chiefly in an attempt to reinstate the patient's hematopoietic system.[3] Hence, the outcome of BMT depends on some factors and one of which is the number and degree of differentiation, of HSCs in graft. [4] To date, the TNC and the number of CD 34 + cells are considered the most common criteria for evaluating the graft content.[5] TNCs include all nucleated cells in the graft, while, CD34 + counts refer to the HSCs that are involved in engraftment and lifelong hematopoiesis only.[6, 7].

TNC and CD34 + counts are done, and used for graft quantity in the developed countries.[8] However, in low resource environment, the two assessments may not be feasible because of the financial and technical constraints that are likely to be encountered.[9] Therefore, it is crucial to identify the best predictor and cost-effective variable to augment the patient's status and resource utilization in such environments.[10]

The aim of this study is to assess the correlation between TNC and CD34+ cell counts and their impact on the graft outcomes in LMICs. This study will seek to determine which of the cell count parameter is more useful in the prediction of engraftment success, incidence of GVHD and survival rates,

Considering the realities of operating in developing countries, this study's goal is to offer practical recommendations for the effective use of allogeneic BMT

by identifying the most informative and resource-friendly cell count parameter. Thus, we want to enhance the survival rate and facilitate the use of allogeneic BMT in centers with limited resources.

Methodology

This is a retrospective design study was conducted at a Gambat Institute of Medical Sciences between 2nd, January 2019 to 1st, February 2024.

The study cohort includes 84 patients who underwent HSCT, 44 patients in the CD34 group and 40 patients in the TNC group. This study was performed to compare demographic and clinical characteristics, graft characteristics, GVHD incidence, engraftment outcomes, and survival data between CD34 selected stem cell transplantation and TNC transplantation. The patients included in the study had been diagnosed with SAA, BTM, POEMS, beta-thalassemia major, AML, PNH, MM and Fanconi's anemia. All patients are fully HLA matched with our donors in this series. Patients with partial HLA matching and incomplete medical records were excluded from the study. The time to engraftment is defined as the first of three consecutive days with an ANC of more than 500/μL.[11] GVHD is an ordinary and potentially life-threatening condition, which is scored according to specific clinical classification.[12]

Recipients were selected according to the compatibility with HLA. Most of them were Sibling donors followed by matched unrelated donors. Regarding the donor, the age varied between 4 to 40 years. Participants in the study signed a consent form and the study was done with the approval of the ethical committee and with the principles of the Belmont report.

Information on TNC and CD34+ cell counts was retrieved from the records of the laboratory. The corrected Total Nucleated Cell (TNC) count was calculated using the following formula:

Volume of the BMH bag X wbc counts of BMH bag/weight of the patient X100) X 45/Neutrophil %

Here's a step-by-step breakdown of the calculation:

Volume of the BMH bag: The total volume of the bone marrow harvest (BMH) bag collected during the procedure.

WBC counts of the BMH bag: The white blood cell count within the BMH bag, indicating the number of nucleated cells present.

Weight of the patient: The body weight of the patient in kilograms, used to standardize the TNC values.

Neutrophil %: The percentage of neutrophils in the white blood cell count, which is used to adjust the TNC value to account for the proportion of neutrophils.

By using this formula, the TNC values are standardized based on the recipient's body weight, allowing for more accurate comparisons across different patients. The final result provides a corrected TNC value that accounts for variations in neutrophil percentages and body weight, ensuring that the TNC count is appropriately normalized. One year and three-year overall survival rates, 1-year and 3-years disease-free survival rates, median follow up were also noted.

Patients taking myeloablative, and non-myeloablative regimen are selected based on our diagnosis and the clinical status of the patient. It is determined by our treating physicians according to the institutional protocols.

Grafts were prepared and administered according to the standard procedure. The grafts used in CD34 group are CD34-selected stem cell transplant where the patients in TNC group received unmanipulated TNC transplant. Graft characteristics for corrected TNC count, CD34 counts, and infusion volume are noted.

Data were analyzed using statistical package for social sciences (SPSS) version 26.0. The patient and transplant characteristics were described using frequency distributions. The Kaplan-Meier test was used to compare overall survival between TNC and CD34.

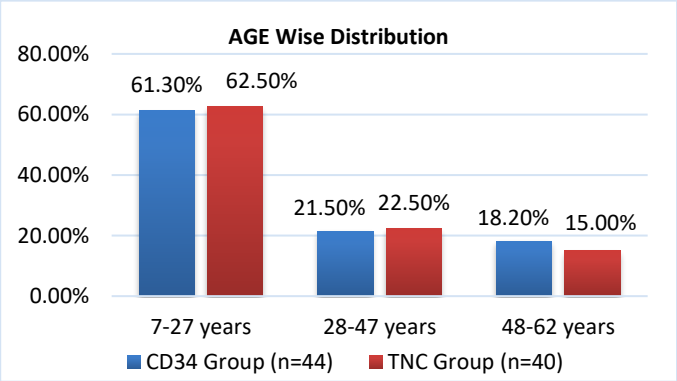
The study was approved by the institutional review board (IRB) of Gambat Institute of Medical Sciences. Patients' consent was sought from all the patients, and their legal representatives where they were below the legal age. The study followed the guidelines of the Declaration of Helsinki.

Thus, this study will use sound statistical techniques and meet the highest ethical standards to offer a comparative analysis of the effects of TNC and CD34+ cell counts on graft outcomes in allogeneic BMT with an emphasis on

the enhancement of practices in the context of resource-constrained environments.

Results

The study comprised of 84 patients, of which 55 were male and 29 were female, with the mean age in CD34 and TNC group were 35.2 years, and 36.1 years (p=0.68), respectively with similar distribution according to prescribed range of ages for each gestation period within the groups itself as well (p=0.56). A similar gender distribution was found in the TNC group, with 65.9% and 65.0% males respectively (p=0.95). Proportions of patients with severe aplastic anemia, beta thalassemia minor, POEMS syndrome, beta thalassemia major, acute myeloid leukemia paroxysmal nocturnal hemoglobinuria multiple myeloma and Fanconi anemia were amounted up between the both groups regarding diagnoses (p=0.62).



Myeloablative conditioning was received in 72.7% of the CD34 group and 67.5% of the TNC group (p=0.37). Most patients in both groups received grafts from related donors (81.8% for the CD34 group and 85.0% for TNC, P=0.48), and all patients had fully HLA-matched donors.

There was no significant difference in the graft characteristics between groups A CD34 count of 5.1 x 10^6/kg in the CD34 group and corrected TNC count was 3.5 x 10^8/kg in the same group compared with a CD34, it is were no differences among both groups (p=0.75). Both groups had similar infusion volumes - 250 mL in the CD34 group and 245 mL in the TNC group (p=0.62). Time to neutrophil and platelet engraftment was also comparable between the groups (p=0.58 and p=0.66, respectively).

Rates of graft-versus-host disease (GVHD) were similar in both groups. The incidence of acute GVHD (Grade I-II) was 22.7% for the CD34 group and 35.0% in TNC groups, while chronic GVHD occurred at a rate of 18.2% of the CD34 group and 25.0% of the TNC group (p=0.42). A greater percentage of patients in the CD34 cohort also sustained longer median GVHD-free survival (59.1% vs. 40.0%; P=0.09).

Table I: Demographic and Clinical Characteristics of Patients

Characteristic	CD34 Group (n=44)	TNC Group (n=40)	p-value
Age (years), Mean ± SD	35.2 ± 10.5	36.1 ± 9.8	0.68
Age (years)			
7-27 years	27 (61.3%)	25 (62.5%)	
28-47 years	9(20.5%)	9 (22.5%)	0.56
48-62 years	8 (18.2%)	6 (15.0%)	
Gender			
Male	29 (65.9%)	26 (65.0%)	0.95
Female	15 (34.1%)	14 (35.0%)	
Diagnosis			
Severe Aplastic Anemia (SAA)	24 (54.5%)	21 (52.5%)	
Beta Thalassemia Minor (BTM)	1 (2.3%)	0	
POEMS	0	1 (2.5%)	
BTM Major	10 (22.7%)	10 (25.0%)	
Acute Myeloid Leukemia (AML)	1 (2.3%)	1 (2.5%)	0.62
Paroxysmal Nocturnal Hemoglobinuria (PNH)	1 (2.3%)	2 (5.0%)	
Multiple Myeloma (MM)	5 (11.4%)	4 (10.0%)	
Fanconi Anemia	2 (4.5%)	1 (2.5%)	
Conditioning Regimen			
Myeloablative	32 (72.7%)	27 (67.5%)	0.37
Non-Myeloablative	12 (27.3%)	13 (32.5%)	
Donor Type			
Related	36 (81.8%)	34 (85.0%)	0.48
Unrelated	8 (18.2%)	6 (15.0%)	
HLA Match			
Fully	44 (100%)	40 (100%)	0.53
Partial	0	0	

Engraftment outcomes were similar with 86.4% successful engraftment in the CD34 group versus 80.0% of patients who successfully gained neutrophil recovery by post infusion for the TNC product (p=0.42). The percentage of primary graft failure was 9.1 % in the CD34 group and 15.0% in the TNC groups (p=0.42) while secondary graft failure occurred at a rate of 4.5% of CD34 group vs. 5.0% of TNC group with (p=0.91) respectively. The CD34 group had a median time to

engraftment of 16 days, and the TNC group also required about as long (17 days, p=0.55).

Table II: Graft Characteristics.			
Characteristic	CD34 Group (n=44)	TNC Group (n=40)	p-value
Corrected TNC Count (x10	3.5 (1.2-6.8)	3.4 (1.1-6.5)	0.75
CD34 Count (x10^6/kg)	5.1 (2.0-8.0)		
Infusion Volume (mL)	250 (50)	245 (55)	0.62
Time to Neutrophil Engraftn (days)	15.3 ± 3.4	16.1 ± 3.6	0.58
Time to Platelet Engraftmer (days)	20.5 ± 4.2	21.0 ± 4.5	0.66

Table III: Incidence of Graft-versus-Host Disease (GVHD)			
GVHD Type	CD34 Group (n=44)	TNC Group (n=40)	p-value
Acute GVHD (Grade I-	10 (22.7%)	14 (35.0%)	0.18
Chronic GVHD	8 (18.2%)	10 (25.0%	0.42
GVHD Free	26 (59.1%)	16 (40.0%	0.09

Table IV: Engraftment Outcomes			
Outcome	CD34 Group (n=44)	TNC Group (n=40)	p-value
Successful Engraftment	38 (86.4%)	32 (80.0%)	0.42
Primary Graft Failure	4 (9.1%)	6 (15.0%)	0.42
Secondary Graft Failure	2 (4.5%)	2 (5.0%)	0.91
Median Time to Engraftm (days)	16 (12-20)	17 (13-21)	0.55

Table V: Overall Survival and Disease-Free Survival			
Survival Metrics	CD34 Group (n=44)	TNC Group (n=40)	p-value
1-year Overall Survival	35 (79.5%)	28 (70.0%)	0.31
3-year Overall Survival	30 (68.2%)	24 (60.0%)	0.40
1-year Disease-Free Rate	33 (75.0%)	26 (65.0%)	0.33
3-year Disease-Free Rate	28 (63.6%)	22 (55.0%)	0.44
Median Follow-Up (mo	24 (12-36)	22 (12-34)	0.58

Rates of overall survival, and disease-free survival were similar between the groups as well. The 1-year overall survival was 79.5% in CD34 group and 70.0% in TNC group (p=0.31), whereas the 3-year overall survival rates were 68.2%, in the CD34 group and 60.0% in the TNC group (p=0.40). The 1-year disease-free survival rates were respectively 75.0% and 65.0% in the CD34 vs. TNC groups (p=0.33), whereas corresponding values for three years were similar, at a rate of treatment success (63.6% in CD34 group vs. 55.0% in TNC group (p=0.44). There were no statistically significant differences with respect to the median follow-up durations (24 months for CD34 and 22 months for TNC, p=0.58).

Discussion

The demographic characteristics, clinical features of recipients and donors as well as transplantation related factors were comparable in patients receiving CD34-selected and TNC transplants.

The demographic and clinical characteristics of the patients were well-matched between the CD34 and TNC groups. The mean age and gender distribution of the groups were comparable, as has been identified in other studies where patient demographics have had no significant influence on outcomes between the two. That the diagnoses were similarly distributed in both groups, moreover demonstrates its construction as an adequate study design capable of reducing confounders to a minimum.[13]

Both groups experienced similar conditioning and there was a slightly but not significantly higher prevalence of myeloablative regimen use in the CD34 group Similar to other reports, the conditioning regimen type did not markedly affect CD34 vs. TNC graft outcomes. The majority of patients in both groups underwent HSC transplantation from related donors, which is standard practice and has been documented as well.[14]

There were no differences in graft characteristics, including corrected TNC count and CD34+ count for the recipient weight, and new-to-cryopreservation AUC volume between groups. This is consistent with earlier studies in which CD34-selected and TNC grafts showed similar engraftment features, no significant difference was detected in engrafting, and survival results.[15]

Rate of acute and chronic GVHD was similar for both groups. In the CD34 group, 22.7 % had acute GVHD and 18.2% chronic; in contrast to TNC group where there were two times higher incidences of both grade I/II AGVHD (35.0%) and chronic GHVD (25.0%). This observation comes in agreement with other studies which showed equal rates of GVHD for CD34 versus TNC

grafts. While the percentage of patients in the CD34 group remaining GVHD-free was greater, this almost reached statistical significance (P=0.06) and so provides a potential trend worthy of further study.[16]

The engraftment results were not significantly different between the groups, with successful engraftments in 86.4% of patients treated with CD34 and 80.0% of patients given TNC. This is in accordance with previous studies, which also found no statistically significant difference between engraftment failure between either CD34-selected, and TNC grafts. Primary and secondary graft failure rates were similar between the groups, which reinforces their comparability in terms of how reliably they engraft.[17]

Both the groups had similar overall survival and disease-free survival rates. In addition, the 1-year and 3-year OS rates were marginally superior in CD34 group but without statistical significance which was again consistent with previous studies that reported comparable survival results between both graft sources. Similarly, the 1-year and 3-year disease-free survival rates between these different graft sources were not dissimilar meaning that there was no distinct advantage of one graft source over another on long-term disease control.[18]

Conclusion

Our study demonstrates, for the first time, that corrected TNC-based grafts yield comparable outcomes to CD34-selected products in allogeneic transplantation, with equivalent engraftment kinetics, GVHD incidence, and survival rates. In resource-limited settings where CD34 selection technology is unavailable, TNC quantification emerges as a clinically validated and cost-effective alternative that does not compromise therapeutic efficacy. While larger multicenter studies with extended follow-up could further validate these findings across diverse patient subgroups, our results provide robust evidence supporting the adoption of TNC-based protocols in transplant programs operating under technological constraints. This approach ensures equitable access to curative transplantation while maintaining rigorous outcome standards.

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