RESEARCH ARTICLE

Dynamics of immune reconstitution in post-allogeneic haematopoietic stem cell transplant patients and effect of potential factors from Pakistani cohort

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Abstract

Objective: To determine the dynamics of immune reconstitution by measuring lymphocyte subsets at periodic intervals after allogenic haematopoietic stem cell transplant (HSCT) and impact of potential factors influencing immune reconstitution.

Methodology: This retrospective study was conducted at the Department of Immunology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from August 2023 to January 2024, and comprised data of patients regardless of age and agender with haematological or immunological diseases who underwent allogeneic haematopoietic stem cell transplant between January 2017 and December 2023. Immune reconstitution was measured using lymphocyte subset analysis by flow cytometry (BD Multitest™ 6-color TBNK kit, USA) on BD FACS Canto™ II at 3-, 6-, and 12-months post-transplant.

Results: Of the 100 patients with mean age 8.61±8.82 years (range: 0.3-53 years), 63(63%) were males and 37(37%) were females. Lymphocyte subsets demonstrated significant progressive reconstitution across 3-, 6-, and 12-month intervals post allo-HSCT (Friedman test, $p < 0.01$ for ALC, $CD3^+$, $CD4^+$, and $CD19^+$). At 12 months, reconstitution rates were: $CD3^+$ 71%, $CD4^+$ 68%, $CD8^+$ 86%, $CD19^+$ 76%, and NK 73%. NK and $CD8^+$ T cells recovered rapidly, while $CD4^+$ and $CD19^+$ subsets achieved reconstitution later. Paediatric patients demonstrated significantly higher $CD4^+$ and CD8+ reconstitution compared to adults ($p < 0.05$). Higher stem cell dose was significantly associated with $CD4^+$ and NK cell reconstitution ($p = 0.016$, OR = 0.88; 95% CI: 0.78 - 1.00 and $p = 0.040$, OR = 0.83; 95% CI: 0.71 - 0.97, respectively). Myeloablative conditioning was associated with higher $CD19^+$ B-cell reconstitution compared to RIC ($p = 0.009$, OR = 2.57).

Conclusion: Lymphocyte subset recovery following allogeneic haematopoietic stem cell transplantation (HSCT) occurs at varying rates, making it essential to monitor these subsets periodically, typically at 3-, 6-, and 12-months post-transplant, to tailor intervention strategies like vaccination schedules. Faster reconstitution of T-cell subsets has been linked to higher stem cell doses and younger patient age.

Keywords: Conditioning regimen, Hematopoietic stem cell transplantation, Immune reconstitution, Lymphocyte subset.

(JPMA 76: 1472; 2026) **DOI: https://doi.org/10.47391/JPMA.30795**

Introduction

Allogeneic HSCT is a well-established curative approach for treating refractory haematological cancers and various benign disorders, establishing a new lymphohaematopoietic system in patients with abnormal haematopoiesis or immune dysfunction.[1] It involves the transfer of healthy hematopoietic stem cells (CD34^+) from a human leukocyte antigen (HLA) compatible donor to patients with neoplastic, dysfunctional, or depleted bone marrow.[2] Approximately 50,000-60,000 HSCT procedures are conducted globally each year,[3] with 9 individuals per 10 million population undergoing HSCT in Pakistan in 2016.[4]

The success of allo-HSCT hinges on immune reconstitution, with the rate and quality of reconstitution being pivotal for clinical outcomes post-transplantation as donor immune cells within the graft contribute to anticancer GVL effects, protect against infections, mitigate GvHD, and correlate with improved overall survival due to reduced infection-related mortality.[5] Major complications of HSCT include infections, relapse, GvHD and to a lesser extent, autoimmune diseases and secondary malignancies, attributable in part to immune deficiency or dysregulation.[6] An optimal graft should effectively reconstitute haematopoiesis and immune function, establish donor chimerism and trigger a graft vs leukaemia (GVL) response while minimizing drastic graft-versus-host disease (GvHD).[7] Immune reconstitution is

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Submission complete: 19-04-2025 **First Revision received:** 22-07-2025

Acceptance: 30-05-2026 **Last Revision received:** 29-05-2026

the process through which the immune system regenerates and restores functionality following immune suppression or depletion, enabling defence against pathogens and maintenance of immune balance.[8]

Despite advancements, significant complications still impact both survival and quality of life after HSCT. Immune reconstitution post-HSCT is impacted by patient and transplant-related factors such as disease type, recipient age, source of stem cells, donor type, conditioning regimen, serotherapy and strategies for GvHD and viral infection prevention and treatment.[9] However, predicting clinical outcomes based on immune parameters remains difficult due to inconsistencies in lymphocyte count thresholds, differences in the timing of post-HSCT blood sampling and variability among patient populations in different studies.[10]

The objective of this study is to determine the dynamics of immune reconstitution by measuring lymphocyte subset at periodic intervals after allogeneic HSCT and the impact of effect modifiers, influencing immune reconstitution. This study aims to provide contemporary insight relative to the anticipated population of Pakistan, rather than consulting international institutions and guidelines, to make better interventional choices for post-Allogenic HSCT care. As far as current literature indicates, this appears to be the first study of its nature carried out in Pakistan.

Methodology

The single-centre, retrospective cohort study was conducted from August 2023 to January 2024 at the Department of Immunology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, and clinical data was retrieved from the Armed Forces Bone Marrow Transplant Centre (AFBMTC), Rawalpindi, after approval from the institutional ethics review committee. Data was extracted for patients regardless of age and gender with haematological (malignant and non-malignant) or immunological diseases for which allo-HSCT was clinically indicated or considered imperative for cure, and who underwent allo-HSCT between January 2017 and December 2023, excluding autologous HSCT patients, those with lymphocyte subset analysis outside specified follow-up intervals, individuals undergoing re-transplantation, cases with mixed donor chimerism (<95% donor cells) at 12 months post-HSCT, HIV-positive individuals and patients receiving donor lymphocyte infusion (DLI). The sample size was calculated using the World Health Organization (WHO) formula for comparing two proportions in OpenEpi 3.01.[11,12] A two-sided significance level of 0.05 and a statistical power of 80% were applied, assuming equal group sizes. The expected outcome proportions were based on reported immune reconstitution rates in post-HSCT patients with severe combined immunodeficiency (SCID).[13] The minimum required sample size thus calculated was 48; however, the sample size was inflated to cover all eligible patients who met the inclusion criteria to enhance statistical precision and generalizability. Due to clinical and logistical reasons, 3-month lymphocyte subset data were available for 41 of 100 patients; 6- and 12-month data were each available for 99 patients; and only 39 patients had complete data across all three time points, forming the repeated-measures analysis cohort.

Immune reconstitution of lymphocyte subset was evaluated by multicolor flow cytometry at three predefined post-transplant intervals i.e. 3 months (±2 weeks), 6 months (±3 weeks), and 12 months (±4 weeks), percentages and absolute numbers of lymphocytes, CD3^+ T cells, CD4^+ T cells and CD8^+ T cells, CD19^+ B cells and CD〖16/56〗^+ NK cells gated for all nucleated CD45^+cells were enumerated (BD Multitest™ 6-Color TBNK kit (BD Biosciences and Company, San Jose, CA, USA)) on the BD FACS Canto™ II (Fluorescence-Activated Cell Sorting) flow cytometer (BD Biosciences and Company, San Jose, CA, USA) using respective conjugates (CD3 Fluorescein Isothiocyanate (FITC) / CD16 Phycoerythrin (PE) + CD56 PE / CD45 Peridinin Chlorophyll Protein–Cyanine 5.5 (PerCP-Cy5.5) / CD4 Phycoerythrin–Cyanine 7 (PE-Cy7) / CD19 Allophycocyanin (APC) / CD8 Allophycocyanin-Cyanine 7 (APC-Cy7)) in a 3 ml Ethylenediaminetetraacetic acid (EDTA) anticoagulated blood sample. Cutoff values for immunophenotypic lymphocyte reconstitution at 12 months post-allo-HSCT were defined using absolute count thresholds derived from established HSCT studies by de Koning et al. (2016) and Park et al. (2015), as follows: $\geq 1.50\times10^9$/L for total lymphocytes, $\geq 0.52\times10^9$/L for $CD3^+$ T cells, $\geq 0.47\times10^9$/L for $CD4^+$ T cells, $\geq 0.20\times10^9$/L for $CD8^+$ T cells, and $\geq 0.15\times10^9$/L for $CD19^+$ B cells.[14, 15] Successful immune reconstitution was defined as achieving or exceeding respective absolute count thresholds for each lymphocyte subset at 12 months post-HSCT. Patients who failed to reach these thresholds were classified as non-reconstituted.

Myeloablative conditioning (MAC) without total body irradiation (Non-TBI) involves high-dose chemotherapy, was administered to 75 patients using primarily alkylating agents and immunosuppressants, including, Fludarabine (Flu), Busulfan (BU), Cyclophosphamide (Cy), Thymoglobulin (Thy), Treosulfan (TREO), Thiotepa (Thio), Etoposide (Etipo). The MAC regimens (Flu/BU/Cy/Thy

(n=42), Flu/BU/Thy (n=13), BU/Cy/Thy (n=6), Flu/BU/Cy (n=5), BU/Cy (n=4), Flu/Cy/Thy/TREO (n=1), Flu/BU/Cy/Thio (n=1), Flu/Thy/TREO/Thio (n=1), Flu/Cy/Thy/TREO/Thio (n=1), and Flu/Cy/Thy/TREO/Etipo (n=1), lead to profound and prolonged cytopenia lasting up to 21 days, thereby requiring stem cell transplantation for haematologic recovery. The remaining 25 patients received reduced intensity conditioning (RIC; n=24), which employs lower chemotherapy doses producing milder myelosuppression and fewer regimen-related toxicities, or no conditioning (n=1).

Graft-versus-host disease (GvHD) prophylaxis included Calcineurin Inhibitor-Based Regimens including Cyclosporine A (CsA), Methotrexate (MTX), and Mycophenolate Mofetil (MMF) in different combinations (CsA, CsA/MTX, CsA/MMF, and CsA/MTX/MMF) and Post-Transplant Cyclophosphamide (PT-Cy) Based Regimen. Details are summarized in Table III. Cytomegalovirus (CMV) reactivation was defined as CMV DNAemia ≥500 copies/mL, detected by quantitative PCR on peripheral blood, with weekly surveillance from day +28 to +100 post-HSCT as part of preemptive monitoring.

Data was analyzed using SPSS (Statistical Package for the Social Sciences) version 27. The Shapiro–Wilk and Kolmogorov–Smirnov tests were employed to assess the normality of the data. Since data was non-parametric so we computed Independent-sample Kruskal-Wallis test with post-hoc testing was done using Dunn's test with Bonferroni correction to compare the medians of absolute lymphocyte subset count across 3-, 6- and 12-months post-HSCT intervals and to evaluate the nature of differences between the groups, respectively. Within-subject comparisons of immune cell count across time points were performed using the Friedman test (non-parametric repeated measures ANOVA), followed by Bonferroni-adjusted Wilcoxon signed-rank tests for post hoc pairwise analysis. Stem cell dose ($CD34^+ \times 10^6$/kg) was compared between reconstituted and non-reconstituted groups using the Mann–Whitney U test. Effect size was quantified using the rank-biserial correlation (r_β) and interpreted according to Cohen's criteria as small (~0.1), moderate (~0.3), or large (≥0.5).[16] In addition, binomial logistic regression was performed to evaluate whether stem cell dose predicted immune reconstitution status of $CD4^+$ T cells and $CD16^+/56^+$ natural killer (NK) cells. Results were expressed as odds ratios (ORs) with 95% confidence intervals.

Chi-square test or Fisher's Exact test was used to determine the association of lymphocyte reconstitution with the categorical variables including age group (children and adults), conditioning regimens (RIC and MAC), GvHD prophylaxis (CsA-based and PT-Cy based), stem cell source (BM and BM+PBSC), HLA matching (MRD and Haploidentical), donor/patient age (children (<14 years) vs adults (>14 years)) and CMV reactivation (yes and no). A 95% confidence level was used, with p-values ≤ 0.05 considered statistically significant. Tukey's box-and-whisker plot was used for a graphical representation of ALC distribution across post-HSCT assessments.

Results

Among the 100 patients included, the mean age was 8.61±8.82 years (range: 0.3–53 years), with a male predominance (63 (63%) males and 37 (37%) females). Detailed patient characteristics, including disease profiles, are presented in Table – I. The mean donor age was 13.52±10.96 years (range: 2–57 years), with 51 (51%) female and 49 (49%) male donors (Table – II).

For HSCT, BM was used as source of stem cell in 85 (85%) cases, BM+PBSC in 11 (11%) cases and PBSC only in 4 (4%) cases with a median total nucleated cell count (TNC) of 5.0

Table-1: Patient characteristics.

Patient characteristics	Number (%)n = 100
Gender	
Male	63 (63%)
Female	37 (37%)
Age (years)	
Children (<14 years)	88 (88%)
Adults (>14 years)	12 (12%)
Mean Age	8.61 ± 8.82 years Range: 0.3-53 years
Diseases	
Thalassemia (β-TM)	48 (48%)
Aplastic Anaemia (AA)	12 (12%)
Fanconi Anaemia (FA)	11 (11%)
Severe Combined Immunodeficiency (SCID)	7 (7%)
Hemophagocytic Lymphohistiocytosis (HLH)	6 (6%)
Acute Lymphoblastic Leukemia (ALL)	4 (4%)
Paroxysmal Nocturnal Haemoglobinuria (PNH)	3 (3%)
Bernard-Soulier Syndrome (BSS)	2 (2%)
Glanzmann Thrombasthenia (GT)	1 (1%)
Congenital Amegakaryocytic Thrombocytopenia (CAT)	1 (1%)
X-linked Thrombocytopenia (XLT)	1 (1%)
Pure Red Cell Aplasia (PRCA)	1 (1%)
Myelodysplastic Syndrome (MDS)	1 (1%)
Leukocyte Adhesion Deficiency (LAD)	1 (1%)
Malignant Infantile Osteopetrosis (MIO)	1 (1%)

Table-2: Donor Characteristics

Donors' characteristics	Number (%) n = 100
Gender	
Male	49 (49%)
Female	51 (51%)
Age (years)	
Children (<14 years)	64 (64%)
Adults (>14 years)	36 (36%)
Mean Age	
13.52 ± 10.96 years	
Range: 2-57 years	
HLA Matching	
Matched related donor	95 (95%)
Haploidentical	5 (5%)

Table-3: Pre-transplant assessment and GvHD prophylaxis

Transplant Details			Number (%) n = 100
Stem Cell Source	BM		85 (85%)
	BM+PBSC		11 (11%)
	PBSC		4 (4%)
Median Stem Cell Dose (IQR: 25^{th} – 75^{th})	5.6×10^6/Kg (4.10 – 8.13)		
Median TNC (IQR: 25^{th} – 75^{th})	5.0×10^8/Kg (4.46 – 5.46)		
Conditioning Intensity	RIC		24 (24%)
	MAC		75 (75%)
	No Conditioning		1 (1%)
GvHD Prophylaxis	Calcineurin	CsA+MTX	59 (59%)
	Inhibitor-Based	CsA+MMF	17 (17%)
		CsA	7 (7%)
	PT-Cy Based		15 (15%)
	No Immunosuppression		2 (2%)

Abbreviations: BM, bone marrow, PBSC, peripheral blood stem cells, TNC, total nucleated cells, IQR, interquartile range, RIC, reduced intensity conditioning, MAC, myeloablative conditioning, GvHD, graft vs host disease, CsA, cyclosporin, MTX, methotrexate, MMF, mycophenolate mofetil, PT-Cy, post-transplant Cyclophosphamide

× 108/Kg (interquartile range (IQR): 1.10) and a median CD34+ cell dose of 5.6 × 106/Kg (IQR: 4.09). There were 2 (2%) patients who underwent transplantation without any immunosuppression. Pre-transplant assessment findings and GvHD prophylaxis regimens are summarized in Table – III.

Differences in absolute lymphocyte subset count between the time points (3, 6 and 12 months) post-HSCT indicated significant variations ($p < 0.05$) in immune cells during these intervals. Post-hoc pairwise comparison Dunn's test, adjusted with the Bonferroni correction, of CD3^+ T cells (Figure – 1b), CD4^+ T cells (Figure – 1c) and CD8^+ T cells (Figure – 1d), CD19^+ B cells (Figure – 1e), demonstrated significant increase from 6 to 12 months post-HSCT ($p < 0.05$). However, no significant variation across allocated intervals post-HSCT was observed in CD 16/56^ + NK cells (Figure–1f) absolute count ($p=0.056$), though the result suggests a borderline trend towards significance, worth further investigation.

Lymphocyte subset data were available for 41 patients at 3 months, and for 99 patients at both 6 and 12 months following allogeneic HSCT, the lymphocyte recovery patterns are shown in Table – IV. At 12 months post-allo-HSCT, the proportion of patients achieving reconstitution based on defined absolute count thresholds was 71% for $CD3^+$, 68% for $CD4^+$, 86% for $CD8^+$, 76% for $CD19^+$, and 73% for NK cells.

Longitudinal analysis using the Friedman test revealed significant changes over time in absolute lymphocyte count (ALC), $CD3^+$ T cells, $CD4^+$ T cells, and $CD19^+$ B cells over the post-transplant time points (3, 6, and 12 months), with all p-values < 0.01, indicating progressive immune reconstitution. In contrast, $CD8^+$ T cells and NK cells did not show statistically significant changes ($p = 0.074$ and 0.086, respectively). Post hoc analysis using Wilcoxon signed-rank tests with Bonferroni correction confirmed that the most substantial increases occurred between 3

Table-5: Summary of lymphocyte reconstitution in all patients

	3 months post HSCTn = 41	6 months post HSCTn = 99	12 months post HSCTn = 99	p-value*	Cutoff Absolute count /L	No. of patients reconstituted (%)
	Absolute count median /µL (range)	Absolute count median /µL (range)	Absolute count median /µL (range)			
ALC	1584(78 – 5733)	2053(504 – 8865)	2912(733 – 12100)	< 0.001	_	_
CD3+ T Cells	1023(58 – 3784)	1331(202 – 5496)	2012(403 – 7139)	< 0.002	≥1.50	71
CD3+ 4+ T Cells	184(8 – 1491)	334(92 – 1499)	635(122 – 2101)	< 0.001	≥0.52	68
CD3+ 8+ T Cells	641(25 – 2293)	816(87 – 4906)	1241(107 – 4582)	0.074	≥0.47	86
CD19+ B Cells	88(0 – 1663)	270(0 – 2413)	454(45 – 4086)	< 0.001	≥0.20	76
CD16+/56+NK Cells	249(13 – 1054)	151(13 – 2305)	194(29 – 1331)	0.086	≥0.15	73

*Friedman test (computed for 39 cases only, with lymphocyte subset analysis data available at 3-, 6- and 12-months post-allo-HSCT)

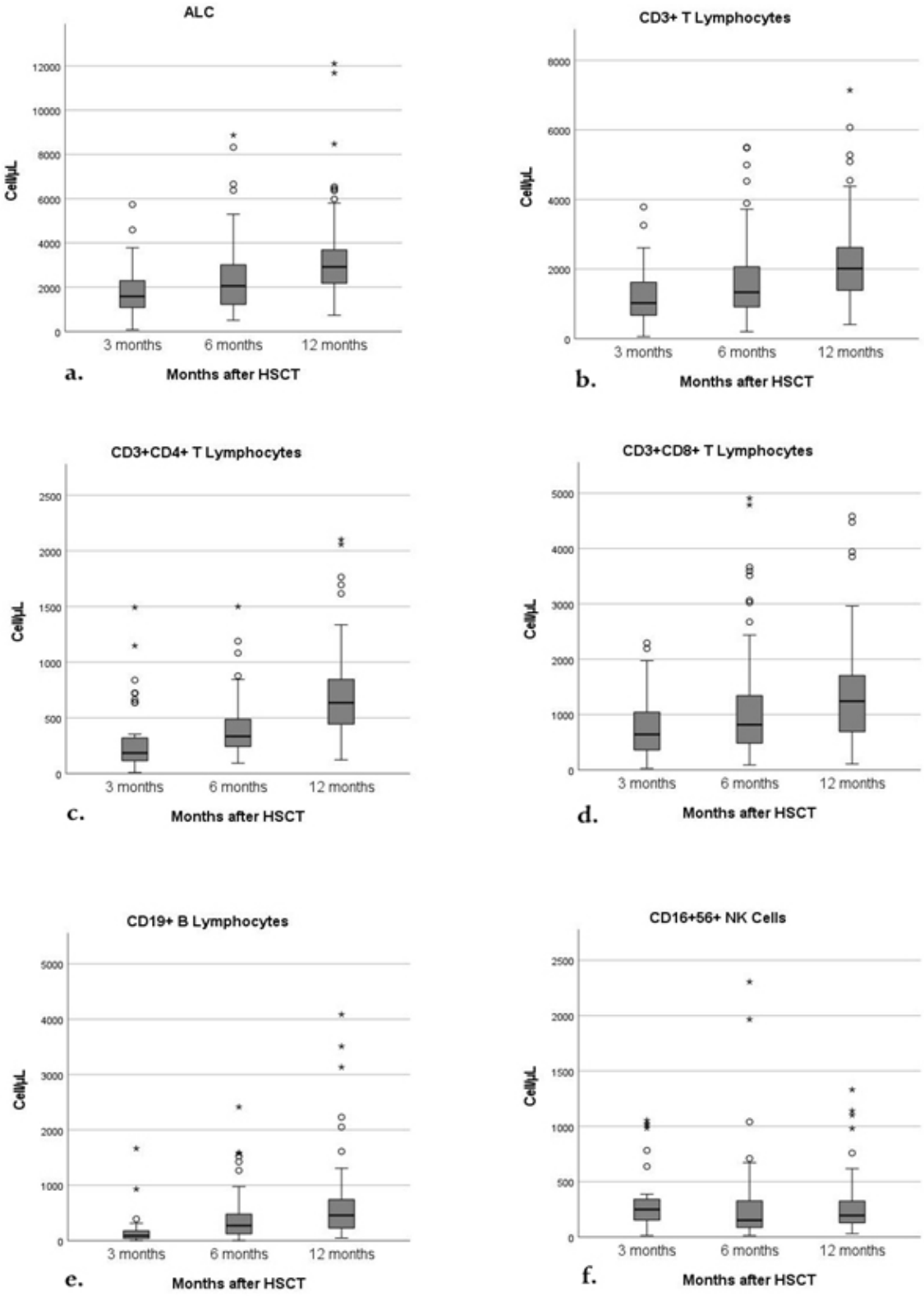

Figure-1: Figure 4.3 (a-f). Distribution of absolute lymphocyte subset count across 3-,6-, and 12-month post-HSCT

Factors		CD3			CD4			CD8			CD19			NK		
		RT	NRT	p-value	RT	NRT	p-value	RT	NRT	p-value	RT	NRT	p-value	RT	NRT	p-value
Conditioning regimens	RIC	14	10	0.102	16	8	0.873	19	5	0.208	14	10	0.009	15	9	0.161
	MAC	56	18		51	23		66	8		62	12		57	17	
GvHD Prophylaxis	CsA-based	56	26	0.218	54	28	0.374	69	13	0.209	64	18	1.000	59	23	0.753
	PT-Cy based	13	2		12	3		15	0		12	3		12	3	
Stem Cell Source	BM	61	24	1.000	61	24	0.104	75	10	0.388	69	16	0.017	64	21	0.386
	BM+ PBSC	10	4		7	7		11	3		7	7		9	5	
HLA	MRD	69	26	0.317	67	28	0.090	83	12	0.436	74	21	0.230	70	25	1.000
	Haploidentical	2	2		1	3		3	1		2	2		3	1	
Donor Age	Children	48	16	0.327	47	17	0.168	57	7	0.535	52	12	0.153	50	14	0.180
	Adult	23	12		21	14		29	6		24	11		23	12	
Patient Age	Children	66	21	0.034	66	21	<0.001	78	9	0.049	69	18	0.143	67	20	0.055
	Adult	5	7		2	10		8	4		7	5		6	6	
CMV reactivation	Yes	43	15	0.525	37	21	0.212	50	8	0.817	46	12	0.467	40	18	0.199
	No	28	13		31	10		36	5		30	11		33	8	

Figure 2: Chi-square and Fisher's Exact Test analysis of effect mediators of Immune cell reconstitution
GvHD, Graft versus Host disease, CMV, Cytomegalovirus, RIC, reduced intensity conditioning, MAC, Myeloablative conditioning, BM, Bone marrow, PBSC, Peripheral Blood Stem Cell, MRD, Matched Related Donor, RT, Reconstituted, NRT, Not reconstituted

and 12 months for ALC (p=0.000), $CD3^+$ (p=0.001), $CD4^+$ (p=0.000), and $CD19^+$ (p=0.000) subsets. $CD4^+$ and $CD19^+$ cells also showed significant gains between 6 and 12 months (p=0.005 and 0.000, respectively), while no significant changes were observed between 3 and 6 months, Table – 4.

There were no statistically significant differences in the stem cell dose between patients who reconstituted and those who were not reconstituted for CD3+ T cells (p = 0.590), CD8+ T cells (p = 0.054), and CD19+ B cells (p=0.363). In contrast, patients in the reconstituted group received significantly (p=0.016) higher doses of $CD4^+$ T cells (median (IQR): 6.10 (5.60)) compared to those who didn't reconstitute (median (IQR): 4.61 (2.85)). Similarly, for $CD16^+/56^+$ NK cells, with significantly (p=0.040) higher median doses in the Reconstituted group (median (IQR): 5.70 (6.00)) versus the Not Reconstituted group (median (IQR): 4.50 (2.86)). The effect size, indicated a moderate relationship, suggesting that stem cell dose has a moderate influence on the likelihood of reconstitution for CD4+ T cells (rb=0.307) and CD16+/56+ NK cells (rb=0.274) specifically.

Binomial logistic regression showed that stem cell dose significantly predicted both $CD4^+$ and NK cell reconstitution at 12 months post-transplant. The model for $CD4^+$ reconstitution was statistically significant (p=0.017; R^2N=0.08), with higher doses of stem cells associated with reduced odds of reconstitution failure (β = −0.13, p= 0.042; OR = 0.88, 95% CI: 0.78 – 1.00). Similarly, the NK cell model showed a stronger association (p=0.004; R^2N = 0.12), where higher stem cell doses significantly decreased the odds of reconstitution failure (β = −0.18, p=0.022; OR = 0.83, 95% CI: 0.71 – 0.97).

No association was found between the reconstitution status of different lymphocyte subsets (CD3+, CD4+, CD8+, CD19+, and NK+ cells) 12-month post HSCT and transplant factors (Disease, GvHD Prophylaxis, Stem Cell Source, HLA Matching, CMV reactivation, and Donor Age) Figure – 2. Although paediatric patients (<14 years) demonstrated significantly higher reconstitution rates compared to adults across multiple immune cell subsets. For $CD3^+$ T cells, n=66 (75.9%) of children reconstituted vs. n=5 (41.7%) of adults (p=0.014; Cramér's V = 0.25, OR = 4.40, 95%CI: 1.26 – 15.33). $CD4^+$ T-cell reconstitution was particularly pronounced (n=66 (75.9%) vs. n=2 (16.7%); p<0.001; Cramér's V = 0.42, OR = 15.71, 95% CI: 3.19 – 77.49). Similar patterns were observed for $CD8^+$ T cells (p=0.027; Cramér's V = 0.22, OR = 4.33, 95% CI: 1.09 – 17.30) and NK cells (p=0.046; Cramér's V = 0.20, OR = 3.35, 95% CI: 0.97 – 11.54), indicating a consistent age-

associated trend favouring immune recovery in paediatric recipients. MAC conditioning was significantly associated with higher $CD19^+$ B-cell reconstitution compared to RIC ($p=0.009$; Cramér's $V = 0.26$). Patients B cells, receiving MAC were 2.57 times more likely to reconstitute (OR = 2.57, 95% CI: 1.27 – 5.18) than who received RIC. This may reflect the enhanced donor-derived haematopoietic engraftment and immune niche clearance associated with MAC protocols.

Discussion

In this study, lymphocyte recovery was evaluated over a 12-month period following allogeneic HSCT in 99 patients with both malignant and non-malignant conditions. The reconstitution of lymphocyte subsets occurred progressively, with each subset demonstrating distinct recovery patterns by the end of the first year. The findings highlight that post-transplant lymphocyte counts are influenced by pre-transplant variables, including patient age, stem cell source, and the administered cell dose.

A study in 2022 retrospectively analyzed the lymphocytes subsets in 111 paediatric post-transplant patients, and concluded that early recovery was noted for CD3+CD8+ cytotoxic T lymphocytes than CD3+CD4+ helper T lymphocytes.[17] Bae et al., 2012 retrospectively analyzed 38 post-transplant recipients and showed rapid recuperation of CD3+CD8+ T lymphocytes when compared to CD3+CD4+ T lymphocytes.[18] Our study is also in agreement to above mentioned studies and showed prior development of cytotoxic T cells in comparison with helper T cells.

A study conducted by Jiang et al at Shanghai China, 2022 showed that older donor has an inferior overall immune reconstitution when compared to younger donor group.[19] González-Vicent et al., 2017 also concluded similar results of faster immune reconstitution of T and B lymphocytes in patients using younger donors.[20] Our results are also compatible with both studies, that suggest recipients from younger donors have rapid and more chances of immune reconstitution in contrast to BMT recipients from older donors.

A study in Spanish population, observed the better and early immune reconstitution in children as compared to adult group.[21] Our results are also in agreement to above mentioned study and showed significant early and better immune reconstitution in children.

One study showed early recovery of CD4+ and CD8+T cell subset in PBSCs compared to BM stem cells,[22] whereas our study highlighted the early recovery of B lymphocytes in recipients when bone marrow derived stem cells are used.

A pilot study in 2023 showed a faster recovery of B cell compartment in patients who underwent MAC compared to RIC.[23] Our study is also in concordance with this study and displayed a faster B cell reconstitution in recipients, who received MAC in contrast to RIC, possibly because MAC regimens induce effective immune ablation, potentially facilitating more robust donor-derived B-cell engraftment and thymic-independent reconstitution.

Elmariah et al., 2021 compared HLA-matched and haploidentical recipients which showed reduced counts of T cells (especially CD4 T lymphocytes).[24] A study by Baumeister et al., 2020 reported the delayed immune reconstitution CD4+ T cells in Haploidentical donors and early CD8+ T cells in fully matched donors.[25] Another Korean study, also deduced the high CD3+CD8+ T cells count in HLA match group.18 Our study is also in consistent with above mentioned studies and showed delayed appearance of CD4+ T lymphocytes in haploidentical transplants and rapid and high counts of CD8+ T lymphocytes in HLA matched patients.

A key limitation of this study is the high variability and presence of outliers in immune cell counts (Figure 1), which were managed using complete-case exclusion and nonparametric, median-based analyses rather than sensitivity testing. Repeated-measures analysis included only 39 patients with complete lymphocyte subset data across all three time points, while lower data availability at 3 months (n=41 vs. 99 at later points) may have reduced statistical power and introduced attrition bias. Furthermore, the absence of associations between immune reconstitution and transplant-related variables should be interpreted cautiously, as limited sample size may have obscured true effects. Further studies are needed to refine cutoff values and clarify the functional significance of lymphocyte recovery patterns.

Conclusion

Lymphocyte subset reconstitution following allo-HSCT occurred at variable rates, which necessitates serial monitoring of lymphocyte subset at 3-, 6- and 12-month post-HSCT. Higher stem cell doses and younger ages were associated with faster reconstitution of T-cell subset. However, more robust analysis and multicenter experiences are needed to elucidate the influence of these factors on transplant outcomes in anticipated population proportion of Pakistan.

Disclaimer: None.

Conflict of Interest: None.

Source of Funding: None.

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AUTHORS' CONTRIBUTIONS:
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MZAJ: Data collection, acquisition and analysis.
MOR: Data interpretation and critical revision.
MA: History taking and statistical input.
MAK: Final approval.
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