https://journal.utripoli.edu.ly/index.php/Alqalam/index eISSN 2707-7179

Original article

Prospective Study of the Recovery of CD4$^+$ T cell Subsets and Their Relationship to the Overall Survival Following Allogeneic Hematopoietic Stem Cell Transplantation

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ARTICLE INFO

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Received*: 03-06-2024*
Accepted*: 26-07-2024*
Published*: 30-07-2024*

Keywords*. Allogeneic Hematopoietic Stem Cell Transplantation, CD4$^+$T Cells, Recovery, Graft Versus-Host-Disease, Naive and Memory Subsets, Overall Survival*

ABSTRACT

The immune recovery (IR) following the allogeneic hematopoietic stem cell transplantation (allo-HSCT) is an advancement procedure that fundamentally correlated to the curative success. It is critical to understand the interfering factors in IR to prevent the HSCT-related mortality. The factors influencing CD4$^+$T cell recovery post (HSCT) are not well totally characterized. This work was conducted to analyze the kinetics of CD4$^+$T cell subsets recovery post HSCT, and to correlate their reconstitution with different factors that may influence the overall survival following HSCT. We prospectively evaluated the clinical outcomes and CD4$^+$ T lymphocyte subtypes regeneration kinetics at different time-points of 22 patients with allogeneic HSCT for malignant and non-malignant diseases from 2007 to 2008. Statistics (means, minimal, and maximal values) were used to describe patient baseline characteristics. Results were presented as absolute count of CD4$^+$ T cells, % of naive and memory subsets, and p-values. Thymus-independent pathways were responsible for the rapid recovery of memory CD4$^+$T cells less than 6 months after HSCT. Thymus-dependent pathways were activated between 6 and 12 months in the majority of patients with increasing counts of naive CD4+ T cell. Furthermore, increasing patient age and chronic GVHD predicted the slow naive T cell recovery and also predicted high memory T cell numbers. The proper CD4 $^+$ reconstitution was associated with younger age, a non-malignant disease and a lower incidence of acute graft-versus-host disease ≥ grade 2. Additionally, the CD4$^+$ T lymphocytes recovery ≥ 200/µl was associated with a higher overall survival. Different factors affected the IR post the allo-HSCT. The CD4$^+$ count ≥ 200/µl was a simple IR predictor of overall survival and better clinical outcome following allogeneic HSCT.

Cite this article. *Elmansorry E. Prospective Study of the Recovery of CD4+ T cell Subsets and Their Relationship to the Overall Survival Following Allogeneic Hematopoietic Stem Cell Transplantation. Alq J Med App Sci. 2024; 7(3):643-655. https://doi.org/10.54361/ajmas.247329*

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a firmly-established yet complicated therapy for different disorders of the hematopoietic system. The noticeable improve in HSCT as a result of the progress in transplant technique and better understanding of the human immune system has led to a progressive raise in the number of HSCTs

performed to a level that, in just half a century, over 1 million HSCTs were performed worldwide, of which about 40 % were allogeneic HSCTs [1]. The present practice of HSCT is rapidly developing and guidelines are continuously updated and revised reflecting the complexity of this process. Despite major advancement in allogeneic HSCT, it is still interfered with the associated complications which represent a major cause of morbidity and mortality among transplanted patients [2]. These complications are due to several factors such as conditioning regimen associated toxicities, prolonged immune deficiency, and organ and tissue damage. Among these complications, Graft-Versus-Host Disease (GVHD) remains a major leading cause of non-relapse mortality following allogeneic HSCT [3].

GVHD is an undesirable immune response triggered by donor immune cells that recognize recipient cells as foreign; resulting in organ and tissue damage [4]. GVHD is a frequent event after allogeneic HSCT. It has been determined that around 50 % of allogeneic HSCT patients develop some degree of GVHD [5]. Disorders caused by GVHD are due to various factors such as organ/tissue damage, immune suppression due to the prolonged use of immunosuppressive drugs with the resulting increased risk of infection [6]. Regardless of the major improvements in the field, little advancement has been reached in GVHD therapy rendering it one of the most frequent causes of transplant-related morbidities and mortalities. GVHD is commonly distinguished in both an acute and chronic form, with symptoms ranging from mild to severe organ damage [7].

Allogeneic HSCT is associated with a crucial period of immune deficiency, predisposing patients to elevated susceptibility of severe life-threatening infections. Therefore, effective recovery of functional immune system is important for beneficial outcome [8]. The innate immune system reconstitutes earlier during the first week's post HSCT, providing primary resistance against bacterial infection. The first innate cells to reconstitute are monocytes, followed by neutrophils [9]. Comparing with the delayed maturation of T and B lymphocytes, which may not complete until the first year after transplantation. Reconstitution of functional immune responses affected by many factors, particularly source of graft, graft versus host disease and/or its preventive therapy. Complete and functional recovery of both innate and adaptive immunity is necessary to limit the susceptibility to infection and to prevent relapse risk after allogeneic HSCT [10,11].

The clinical outcome after allogeneic HSCT depends to large extent on proper T cell recovery which is dynamic and complex procedure. Following allogeneic HSCT, the reconstitution of the T cell pool is achieved by two distinct procedures; A) thymus-independent homeostatic proliferation of donor derived mature T cells, and B) thymus-dependent *de novo* generation of naive T cells from donor HSCs [9,12,13].

Primary lymphoid immunity is regulated by occupant mature naive and memory T cells that instantly undergo hemostatic peripheral expansion to restore the T cell components. Their peripheral expansion is affected by either positive or negative T cell selections, stem cell source, cytokine exposure or T cell receptor stimulation. [14] This thymus-independent pathway is critical for early T cell recovery, as thymus-dependent *de novo* generation of naive T cells from donor HSCs takes at least six to twelve months to arise and influenced by variety of factors such as age, stem cell source and GVHD [15,16]. This procedure results in the appearance of different phenotypically naive T lymphocytes that matured in the thymus, collectively increasing their T cell receptor diversity, which is related to a better clinical outcome [17,18].

The naive T cell compartment ($CD45RA^{+}CD45RO^{-}$ $CD31^{+}$) contains of large number of cell-populations with unique T cell receptors, which potentially proliferate and differentiate into all types of effector and memory progenies upon activation with newly encountered antigens [19].

After allogeneic HSCT, early activation of donor naive T cells is correlated with GVHD, suggested to be a result of large numbers of allo-reactive precursors due to the vast diversity of the naive T-cell receptor repertoire [20-23]. In general, adequate naive T cell regeneration is critical for long term immune activity and tolerance [24, 25], and correlates to evolved overall survival [20,26].

The memory T cell subsets ($CD45\ RA^{-}CD45RO^{+}CD31^{-}$) are able to survive for long time [27-29], have promoted proliferative potential and immune reconstitution capacity, and therefore thought to be the key source for immunological memory [27]. The memory T cell compartment is preserved through maturation from naive T cells [29,30]. This important compartment is depleted in HSCT patients, leading to an urgent recovery of immunological memory from the time of Transplant.

Immunological memory transferred with the graft depends on the graft type, and recovery of memory T cells highly depends on the quality and number of infused memory T cells within the graft [31-33]. This work was conducted to analyze the kinetic of $CD4^{+}$ T cell subsets reconstitution following allogeneic hematopoietic stem cell transplantation (HSCT) and to correlate their recovery with different factors that affect their regeneration and the overall survival post HSCT.

METHODS

Study design

Our Prospective observational study was carried out in laboratory of cellular therapy, Campus Virchow Clinic, Charite' University, Berlin, Germany in the period between January 2007 to January 2008.

Patient cohort

22 consecutive patients (both male and females) of aged between 0.5- 26 years were included in this study. The sample size was estimated on the basis of a single proportion. The underlying disease was acute myeloid leukemia (n=4); acute lymphocytic leukemia (n=7); Wiskott - Aldrich syndrome (n=3); chronic myeloid leukemia (n=1); Fanconi anemia (n=2); myelodysplastic syndrome (n=3); severe combined immunodeficiency (n=1); adrenoleukodystrophy (n=1). In patients underwent reduced-intensity conditioning transplantation, total donor chimerism was assessed from bone marrow aspirates. Genotyping was analysed by short tandem repeat typing using the ABI 310 Genetic Analyser (Applied Bio systems, Inc., Foster City, CA). Alleles specific to donor or recipient were used for chimerism identification. Patients and transplant characteristics are presented in (Table 1).

Conditioning regimen and GVHD prophylaxis

Conditioning regimens consisted of Amsacrine+ fludarabine + cyclophosphamide + total body irradiation (AMS+FLU+CY+TBI, n = 1), busulfan + cyclophosphamide + rabbit antithymocyte globulin (BU+CY+ATG, n = 6), BU+ CY +Melphalan (n=4), BU+CY+FLU (n=2), TBI+VP-16(n=7), CY+ATG (n = 8), BU+FLU (n=2). A total of 16 patients received ATG, Alemtuzumab, n = 2, OKT 3 (n=1)). The GVHD prophylaxis was based on cyclosporine + methotrexate (CsA +MTX, n = 9), cyclosporine A + mycophenolate mofetil (CsA+MMF, n = 6).

Blood samples

Following-transplant fresh blood samples were collected from patients once on day 30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, and day 360 post transplantation. Informed consent was obtained from all patients or their parents**.**
The study protocol was approved by laboratory of cellular therapy, Campus Virchow Clinic, Charite' University, Berlin, Germany.

Flowcytometric analysis for the enumeration of naive and memory CD4+ T cells

Absolute CD4+ cell counts and their subsets were determined using four-color FACS Calibur (Becton Dickinson, USA) flow cytometer according to the manufacturer's instructions**.** A 20 µl Volume of antibodies reagent (either Allophycocyanin (APC)-conjugated anti-CD4(clone SK3)/ Fluorescein (FITC)-conjugated anti-CD45RA (clone L48)/Phycoerythrin (PE)-conjugated anti-CD45RO (clone UCHL1) or PE-conjugated anti-CD31 (L 133.1) was aliquoted into a FACS tubes containing 50 µl of heparinized whole blood, then incubated shortly in the dark place for 15 min at room temperature. Lysis of red blood cells was subsequently carried out by the addition of 450 µl FACS Lysing solution and incubation of samples for a further 15 min at RT. Samples were acquired using a FACS Calibur ((Becton Dickinson) flow cytometer and results were analyzed by FlowJo software (BD).
For each run, appropriate controls were included. Single color-stained beads (compensation beads, BD) were used to create the compensation matrix, and fluorochrome minus one (FMO) control were used for proper gating of cell populations.
Absolute CD4 $^{+}$ cell counts were calculated automatically by Cell Quest program software every month from day 30 until day 360 post transplant. Naive and memory $CD4^{+}$cells were defined according to phenotypic and functional analyses performed previously and were presented as a percentage of total $CD4^{+}$ T cell count.

Statistical analyses

Statistics (means, minimal, and maximal values) were used to describe patient baseline characteristics. Results are presented as mean values of $CD4^{+}$ Lymphocytes, % of $CD4^{+}CD45RA^{+}$, % of $CD4^{+}CD45RO^{+}$, and $CD4^{+}CD31^{+}$ T cells, and *p*-values. Data was analyzed using the Licensed IBM SPSS 24.0 for Windows statistical analysis packages. Chi square test was used to compare categorical variables, while continuous variables were compared using the Mann-Whitney U test. Correlations between given variables were investigated using the nonparametric test of Spearman's rank correlation coefficient. ANOVA was used for related (paired) groups. Survival function was estimated using the Kaplan-Meier method and group differences were compared using the log-rank test. 2-sided P value ≤ 0.05 was considered statistically significant for all tests.

RESULTS

Patient characteristics are described in Table 1. The cohort consisted of malignant and non- malignant diseases (Bone marrow failure syndromes, inborn errors of immunity and inborn errors of metabolism). Graft source was 77% (n=17) bone marrow and 23% (n=5) Peripheral Blood stem cell (PBSC). Seventeen (77%) received their grafts from matched unrelated donors (MUD) and five patients (23%) from matched related donors (MRD). Twenty of the patients (91%) had acute graft versus host disease(aGVHD), range from grade I – II, sixteen patients (73%) had chronic graft versus host disease(cGVHD). GVHD was defined as acute if it occurred before day 100 and chronic thereafter. High -resolution DNA typing for HLA-A, -B, -C, DRB1, and –DQB1were performed in all patients and donors in the transplantation center. In two patients, T cell-replete peripheral blood grafts in combination with low- dose anti-T-lymphocyte globulin was performed to prevent GVHD and graft rejection.

Table 1. Patients and transplant characteristics

Variables	Number (%)
Males **Females**	14(64%) 8(36%)
Patients 'age mean(range)	10.8(0.5-26)
Donor age mean (range)	30(7-50)
Stem cell source Peripheral blood Bone marrow	 5(23%) 17(77%)
T cell- repletion	2(9%) 1x 10^6cells
Acute GVHD (grade I-II) **Chronic GVHD**	20(91%) 16 (73%)
Donor code Matched related donor (MRD) Matched unrelated donor (MUD)	 5(23%) 17(77%)
HLA typing 10/10 9/10 8/10	 15(68%) 4(18%) 3(14%)
Hematological disease Malignant Non-malignant	 13(59%) 9(41%)

HLA= Human leukocyte antigens

Reconstitution of CD4$^+$T Lymphocytes

Absolute count of CD4$^+$ lymphocytes/ml was measured in whole blood every month during first year of follow- up. Counts of CD4$^+$ lymphocytes were presented as median, minimal, and maximal values (Table 2). Median CD4 $^+$T cell numbers were below healthy control levels during the first 6 months post HSCT. Patients with a CD4$^+$ count of less than 0.2 cells/ml were considered profoundly immunosuppressed and were maintained on antiviral, antibacterial and antifungal prophylactic treatment. CD4$^+$ recovery had restored within the normal range by 12 months post HSCT.

Table 2. Recovery of CD4 +T cell /ml after HSCT

Time post HSCT	Median	Minimum	Maximum	Interquartile area
Before Transplantation	0.45	0,02	5,45	0.52
Day 30	0.12	0.02	0.71	0.68
Day 60	0.10	0.01	0.56	0.13
Day 90	0.11	0.01	0.80	0.11
Day 120	0.08	0.01	0.55	0.11
Day 150	0.27	0.06	0.86	0.29
Day 180	0.31	0.05	0.87	0.20

Day 210	0.34	0.06	0.89	0.22
Day 240	0.36	0.06	1.02	0.25
Day 270	0.38	0.07	1.1	0.26
Day 300	0.41	1.01	2.1	0.31
Day 330	0,45	1.1	2.4	0.34
Day 360	0.51	1.2	2.51	0.40

Reconstitution of Naive CD4$^+$T Lymphocytes

Percentages of naive T cells (CD4 $^+$CD31$^+$ / CD4$^+$ CD45RA$^+$) T cells were also low during the first 3 months post HCT and increased gradually after HSCT. Recovery of naive CD4$^+$ T cell numbers after day 100 post HSCT depends on the thymus maturation of T cell precursors and is not a result of peripheral expansion of naive, graft-derived T cells or reversion of memory T cells to a naive phenotype.

Table 3. CD4+CD31+ lymphocytes %during 12months post allogeneic HSCT

Time post HSCT	Mean	Minimum	Maximum
Day 30	**28**	**10**	**49**
Day 60	**39**	**12**	**58**
Day 90	**44**	**13**	**59**
Day 120	**46**	**15**	**62**
Day 150	**49**	**17**	**73**
Day 180	**52**	**18**	**78**
Day 210	**56**	**19**	**81**
Day 240	**58**	**20**	**85**
Day 270	**61**	**22**	**86**
Day 300	**65**	**28**	**88**
Day 330	**68**	**30**	**88**
Day 360	**79**	**41**	**89**

Table 4. CD4+CD45RA+ T cells % during 12months post allogeneic HSCT

Time post HSCT	Mean	Minimum	Maximum
Day 30	**2**	**0**	**16**
Day 60	**3**	**0**	**25**
Day 90	**6**	**0**	**39**
Day 120	**7**	**0**	**42**
Day 150	**13**	**2**	**51**
Day 180	**18**	**3**	**58**
Day 210	**22**	**6**	**62**
Day 240	**30**	**10**	**69**
Day 270	**36**	**12**	**75**
Day 300	**42**	**13**	**79**
Day 330	**47**	**19**	**81**
Day 360	**52**	**22**	**85**

Reconstitution of Memory CD4$^+$ T Lymphocytes

In contrast to the slow recovery of naive T cells, memory T cell (CD4$^+$CD45RO$^+$) percentages were high during the first three months post HSCT). That could be represents homeostatic expansion of graft derived T cells. Furthermore, these results confirm previous observations that T cell numbers are restored in the short-term post HSCT via peripheral expansion of graft-derived mature T cells.

Table 5. CD4+CD45RO+ T cells % during 12months post allogeneic HSCT

Time post HSCT	Mean	Minimum	Maximum
Day 30	**96**	**69**	**99**
Day 60	**95**	**42**	**99**
Day 90	**90**	**40**	**98**
Day 120	**88**	**39**	**95**

Day 150	**83**	**33**	**81**
Day 180	**75**	**30**	**79**
Day 210	**69**	**29**	**72**
Day 240	**66**	**27**	**69**
Day 270	**60**	**21**	**65**
Day 300	**54**	**19**	**61**
Day 330	**47**	**15**	**53**
Day 360	**44**	**13**	**45**

Factors affecting CD4⁺T cells recovery

The factors affecting the function of thymus-dependent and thymus-independent pathways following HSCT were assessed. Reconstitution of $CD4^+$ cells was affected significantly with the age and type of disease (P =0.002 and P =0.003, respectively). Variables associated with $CD4^+$lymphocyte regeneration are described in Table 6 with the categorical variables analyzed.

Table 6. Pre-transplant factors related to CD4+ T cells recovery

Variables	D+100 $CD4^+ \geq 200/\mu L$ P-value
Age (≥15 y vs.15y)	0.002
Disease (malignant vs. non-malignant)	0.003
Source (BM vs. PBSC)	NS
Donor (MRD vs. MUD)	NS

D+100: after day100; BM: bone marrow; PBSC: peripheral blood stem cells; MRD: Matched related donor; MUD: Matched unrelated donor; NS: not significant.

Incidence of aGVHD according to CD4⁺ reconstitution

The 12 months CI of acute GVHD (aGvHD) ≥ grade 2 was higher in the matched unrelated donors, with PBSC and malignant diseases. Inadequate $CD4^+$ regeneration was associated with aGvHD. Furthermore, $CD4^+$ recovery was significant when correlated with the use of the PBSC and MUD (Table 7). There was no significant relationship between type of the malignant disease and relapse with any immune recovery parameter (data not shown).

Table 7. Correlation between aGVHD & CD4+ T cells recovery

Variables	CI %	P-value
Matched related donor **Matched unrelated donor**	23 68	< 0.02
Source Bone Marrow Peripheral blood stem cells	 23 68	< 0.02
Disease Malignant Non- malignant	 65 35	0.03
$CD4^+$ recovery <200/µl ≥ 200/µl	 15 3	0.01

aGvHD: acute graft-versus-host disease; CI: cumulative incidence

Impact of Patient age on Naive CD4⁺T cells recovery

The patients were divided into two groups with the first group ranging from 0.5 to 15 years old and the second group ranging from 16 to 26 years old. The groups were divided in this way simply to have significant numbers within each group since previous reports described a linear decline in naive T cells levels throughout life. The effect of increasing age on naive T cell recovery was analyzed. There was no statistically significant difference between numbers of naive T cells in the two patient groups over the first 6 months post However, following 6months after transplant, there were significantly more $CD45RA^+$ and $CD31^+$T cells in the younger patient group compared to the older patient group (P=0.0005, P =0.001; respectively) (Fig 1&2).

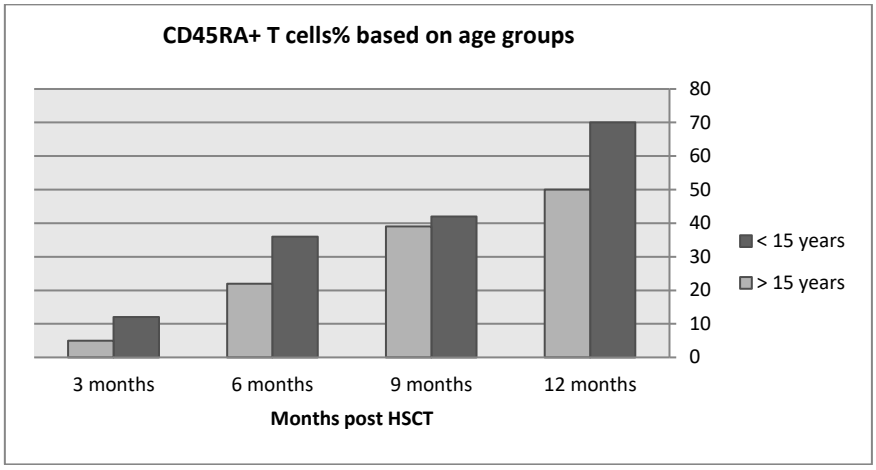

Figure 1. Effect of patient age on and CD4⁺CD45RA⁺ T cell recovery

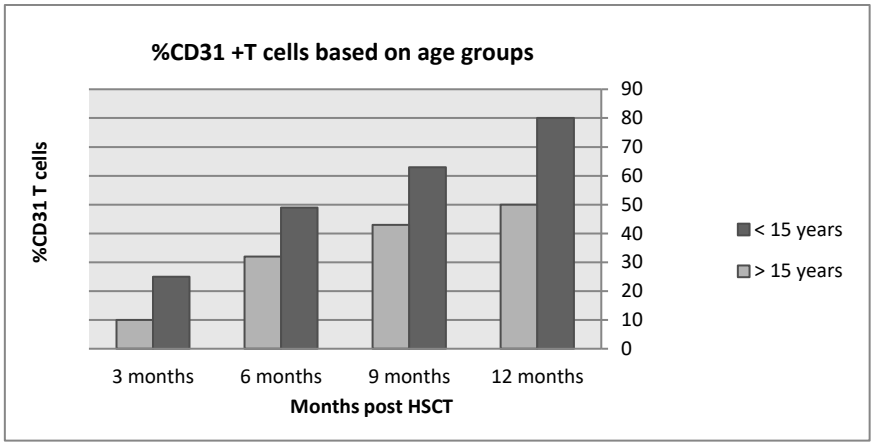

Figure 2. Effect of patient age on and CD4+CD31+ T cell recovery

Impact of Patient age on memory CD4⁺ T cells recovery

This study showed an inverse relationship of patient age with recovery of naive T cells. It is also known that naive T cell numbers decrease and memory T cell numbers increase as part of the normal ageing process. Therefore, the effect of patient age on the recovery of memory T cells after HCT was assessed. Analysis of memory T cell subsets (CD4 ⁺ CD45RO⁺) disclosed that the Percentages of these cells were higher in the older patient group at all-time points. This was statistically significant at 3, 6 and 12 months after transplant (P = 0.02, 0.03 and 0.01 respectively).

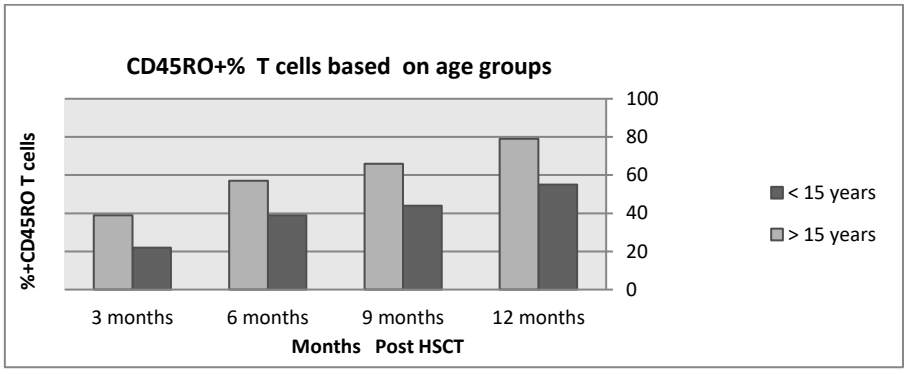

Figure 3. Effect of patient age on and CD4+CDRO+ T cell recovery

Impact of chronic GVHD (cGVHD) on theCD4⁺ T cell subsets recovery

The effects of chronic GVHD (cGVHD) on naive T cell recovery do not become apparent until 12 months after transplant in the majority of patients. %of naive CD4 ⁺T cell at 9 and 12 months post-transplant were significantly

reduced in patients experiencing chronic GVHD compared to patients without cGVHD (P = 0.0016 and P = 0.006, respectively). Furthermore, cGVHD had no significant effect on memory cell reconstitution post HSCT.

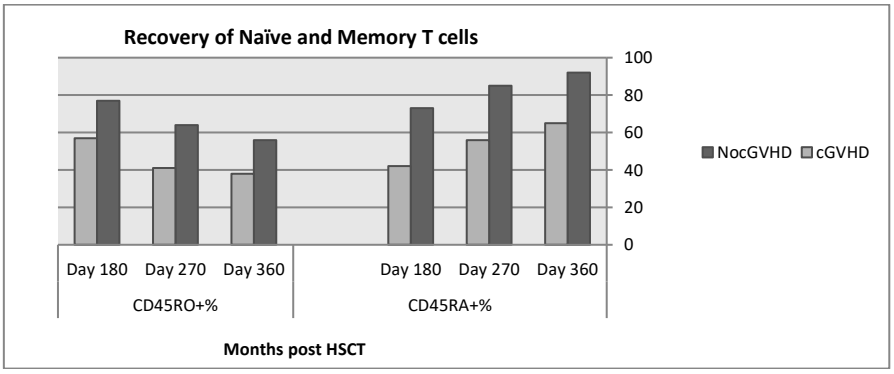

Figure 4. Effect of cGVHD and naive and memory CD4+T cell recovery

Impact of underlying disease on theCD45RA$^+$ T cells recovery

At three months post allogeneic HSCT, Naive T cells reconstitution was significantly higher in patients transplanted for nonmalignant disorders (P = 0.001).

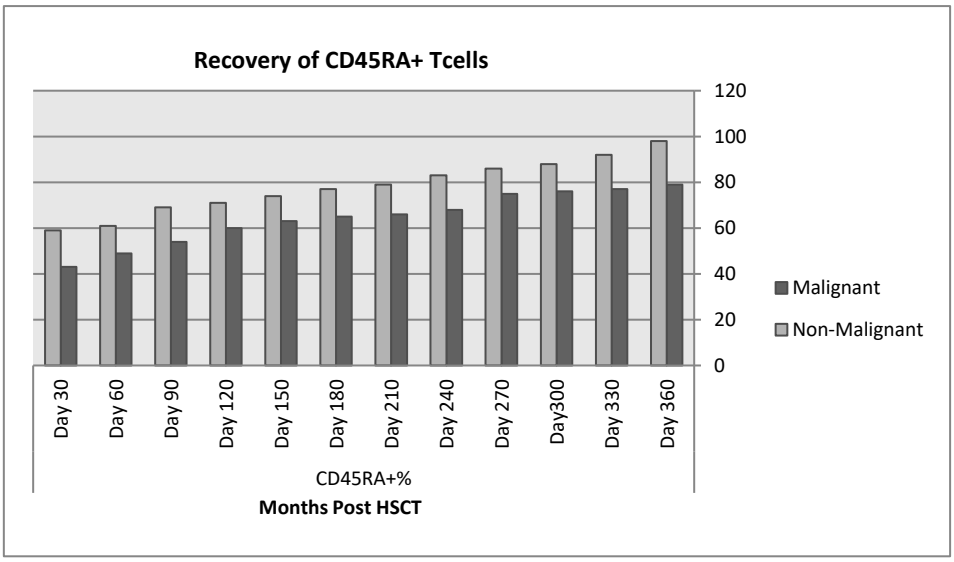

Figure 5. Correlation between underlying disease and CD4+CD45RA+ T cell recovery

Impact of underlying disease on CD4$^+$CD31$^+$ T cells recovery

Following six months post- allogeneic HSCT, CD31$^+$reconstitution was significantly higher in patients transplanted for non- malignant diseases (p≤0.03).

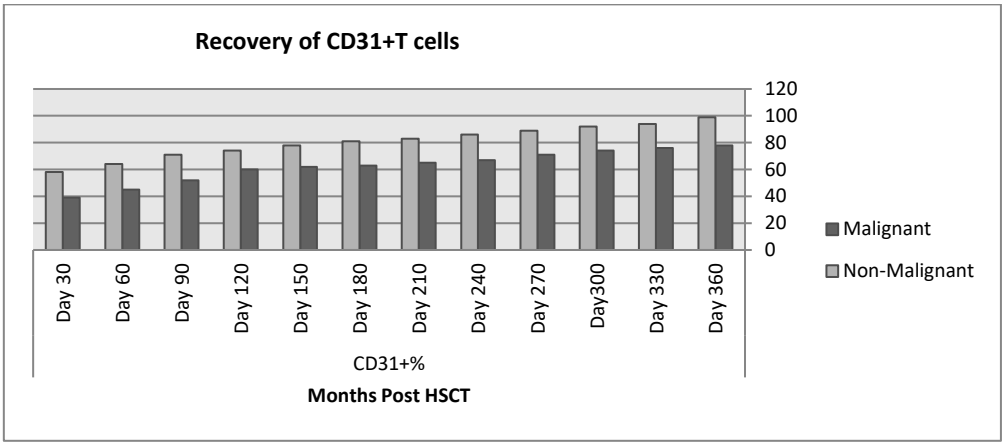

Figure 6. Correlation between underlying disease and CD4+CD31+ T cell recovery

Lymphocyte population's recovery and overall survival CD4$^+$

Over a median follow-up of 12 months, 4 patients died between days +35 and +360. The causes of death were relapse (n = 1), infection (n = 2) and GVHD (n = 1). The factors associated with a higher overall survival and proper clinical outcome were non-malignant diseases and bone marrow as the stem cell source. CD4+ recovery on D+100 was the most important parameters related to higher survival rates.

Table 8. Correlation between CD4+ T cells recovery and overall survival

Variables	Kaplan-Meier	
	%	P-value
Source		
Bone Marrow	77	< 0.01
Peripheral blood stem cells	23	
Disease		
Malignant	58	< 0.01
Non- malignant	41	
CD4$^+$ recovery		
<200/µl	12	0.03
≥ 200/µl	88	

DISCUSSION

Effective regeneration of the acquired immune system after allogeneic HSCT is essential for valuable outcome. The restoration of the virtual empty lymphocyte pool occurs through cytokine driven homeostatic expansion of donor mature lymphocytes and through de novo generation of naive lymphocytes from HSCs precursors. The latter can be monitored by assessing CD45RA+ and CD31+ T cells levels in the Peripheral blood.

Following hematopoietic stem cell transplantation (HSCT), there is a prolonged period of profound immune deficiency, which includes defects in thymopoiesis [34]. This immune deficiency contributes to the high incidence of opportunistic infection, which continues for years after HSCT [35,36].

The etiology of the immune defect is multifactorial. Thymopoietic defects resulting in decreased ability to generate new T cells after HSCT are important since complete immune reconstitution ultimately depends on the generation of new T cells from hematopoietic stem cell (HSC), just as long-term myeloid and erythroid reconstitution depends on HSC engraftment. Transfer of committed progenitors or mature donor-derived T cells may permit short-term immune function. Analyses of patients after HSCT have demonstrated that the presence of immune function at one year or later was correlated with the number of CD4+CD45RA+ naive T cells, suggesting that immune function at later time points is dependent on the ability to generate new T cells [35,37]. T Lymphocytes are generated through two different pathways: thymus dependent and independent [38,39], particularly in the hematopoietic stem cell transplantation setting, Peripheral expansion of T cells can contribute significantly to the composition of the T cell compartment post HSCT [40].

Previously published studies on the frequency of naive CD4+ T cells in blood among pediatric and adult recipients of HSCT suggested that most naive T cells are processed in thymus [41]. Lewin reports on a faster thymic recovery post

HSCT among children indicating that the high residual thymic activity of early childhood might allow for a rapid regeneration of T cells [42].

This work analyzed the CD4+ T lymphocytes reconstitution post allo-HSCT in patients with malignant and non-malignant disorders up to 365 days after the HSCT. Over this 12- month period, the CD4+ T-cells recovery was gradual and each subtype showed different regeneration rates after three months. Our study restates that the CD4+ T cell count following HSCT influences patient survival and is correlated with pre-transplant elements, such as age, source of graft, donor, conditioning regimens, type of disease and GVHD [43,44]. We analyzed naive CD4+ recovery by Flow cytometric assay of CD4+CD31+ or CD4+CD45RA+ CD45RO- T-cell subsets.

This study showed the following; (1) total CD4 +T cells reconstitute to the normal limits in most patients between six and nine months after HSCT; (2) memory T cells are recovered fast (before six months after HCT) with naive T cells recovering later between six months and one year after HSCT; (3) increasing patient age is predictive of slow naive T cell regeneration and increased recovery of memory T cells; (4) patients with chronic GVHD had remarkably lower naive T cell recovery compared to patients with no history of GVHD; (5) patients with malignant disorders had profoundly reduced naive T cell recovery compared to patients with no history of malignancy [45].

The higher naive lymphocyte recovery in younger patients in Our study proved previous studies [41,42] that age alone is considered as critical factor determining the improvement of thymus output to T cell regeneration after HSCT. Additionally, our results reveal that patient age could be the main important factor controlling the achievement of immune recovery following HSCT and whether thymus dependent or thymus-independent pathways participate in the procedure of T lymphocyte recovery after HSCT. The relationship between age and low T cell function has been illustrated in a different of clinical settings [46, 47].

Patients who experienced continuous episodes of chronic GVHD manifested reduced naive T cell recovery. This establishes that the chronic GVHD and/ or steroid therapy and immune-suppressive medications that used in its therapy have counter effects on the peripheral T cell activation and their reconstitution. The thymus damage caused by allo-reactive T cells has a major role in the evolution and pathology of GVHD [48, 49]. Furthermore, our results together with the proof of GVHD-mediated thymus damage show that the thymus is incapable to contribute to T lymphocyte recovery in patients with chronic GVHD following HSCT.

Patients who received their grafts from bone marrow showed higher CD4+ reconstitution in the first months and the donor type play an important role in the early CD4+ recovery [31, 41]. The development of the acute GVHD correlated with reduced CD4+ recovery on D+100. Previous reports indicated that patients with aGVHD showed low CD4 +counts and naive CD4+ percentages up to three months following HSCT [50]. It is well demonstrated that relapse and type of malignant disorder was correlated with inadequate immune recovery and our results proved that the CD4+ compartment recovery was better in pediatric patients with non-malignant diseases [51]. Similar with a previous report, our results confirmed that the CD4+≥ 200/µl on D+100 is a good predictor of the overall survival for pediatric patients undergoing the allo-HSCT and the reduced D+100 CD4+ reconstitution was accompanied with increasing mortality from severe infections and acute GVHD [52,53].

CONCLUSION

In conclusion, this study summarized the CD4+ lymphocyte recovery during first year following HSCT for malignant and non-malignant diseases. The proper CD4 + reconstitution was associated with younger age, a non-malignant disease and a lower incidence of acute graft-versus-host disease ≥ grade 2. Additionally, the CD4+ count ≥ 200/µl was a simple immune recovery predictor of overall survival and better clinical outcome following allogeneic HSCT.

Acknowledgments

All materials and instruments were provided by immunology laboratory Campus Virchow Clinic, Charite' University, Berlin, Germany.

Conflicts of Interest

The authors declare no conflicts of interest.

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https://journal.utripoli.edu.ly/index.php/Alqalam/index eISSN 2707-7179

دراسة استباقية للتعافي المناعي وعلاقته بنجاة المرضى بشكل عام بعد عملية زرع الخلايا الخيفية الجذعية الدموية

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المستخلص

إن التعافي المناعي بعد عملية زرع الخلايا الخيفية الجذعية الدموية هو إجراء متطور يرتبط ارتباطًا أساسيًا بنجاح العلاج. من الأهمية بمكان فهم العوامل المتداخلة في التعافي المناعي لمنع الوفيات المرتبطة بعملية زرع الخلايا الخيفية الجذعية الدموية. العوامل التي تؤثر على تعافي الخلايا التائية الحاملة للمستقبل سي دي 4 بعد عملية زرع الخلايا الخيفية الجذعية الدموية ليست محددة تمامًا. تم إجراء هذا العمل استباقيا لتحليل حركية تعافي مجموعات الخلايا التائية الحاملة للمستقبل سي دي 4بعد عملية زرع الخلايا الخيفية الجذعية الدموية ، وربط إعادة تكوينها بعوامل مختلفة قد تؤثر على البقاء الإجمالي بعد عملية زرع الخلايا الخيفية الجذعية الدموية. لقد قمنا بتقييم النتائج السريرية وحركية تجديد الخلايا التائية الحاملة للمستقبل سي دي 4في نقاط زمنية مختلفة لـ 22 مريضًا خضعوا لزراعة الخلايا الجذعية المكونة للدم من النوع الخيفي لأمراض خبيثة وغير خبيثة من عام 2007 إلى عام 2008. تم استخدام الإحصائيات (المتوسطات والقيم الدنيا والقصوى) لوصف خصائص خط الأساس للمريض. تم تقديم النتائج على أنها عدد مطلق للخلايا التائية الحاملة للمستقبل سي دي4 ونسبة الخلايا الساذجة والذاكرة وقيم .p كانت المسارات المستقلة عن الغدة الزعترية مسؤولة عن التعافي السريع لخلايا الذاكرة التائية الحاملة للمستقبل سي دي4 بعد أقل من 6 أشهر من زراعة الخلايا الجذعية المكونة للدم. تم تنشيط المسارات المعتمدة على الغدة الزعترية بين 6 و 12 شهرًا في غالبية المرضى مع زيادة في عدد الخلايا التائية الحاملة للمستقبل سي دي4 الساذجة. علاوة على ذلك، فإن زيادة عمر المريض ومرض الزراعة ضد المضيف المزمن تنبأ بالتعافي البطيء للخلايا التائية الساذجة وتنبأ أيضًا بأعداد عالية من الخلايا التائية للذاكرة. ارتبطت الإعادة السليمة لتكوين الخلايا التائية الحاملة للمستقبل سي دي 4 بالعمر الصغير والمرض غير الخبيث وانخفاض معدل الإصابة بمرض الزراعة ضد المضيف الحاد ≤ الدرجة 2. بالإضافة إلى ذلك، ارتبط تعافي الخلايا الليمفاوية التائية الحاملة للمستقبل سي دي 4بتركيز ≤200 ميكرو لتر ببقاء أعلى بشكل عام. أثرت عوامل مختلفة على التعافي المناعي بعد زراعة الخلايا الجذعية المكونة للدم. كان عدد خلايا التائية الحاملة للمستقبل سي دي 4بتركيز ≤200 ميكرو لتر مؤشرًا بسيطًا للتنبؤ بالبقاء على قيد الحياة بشكل عام والنتائج السريرية الأفضل بعد زراعة الخلايا الجذعية المكونة للدم من الخلايا الجذعية المتماثلة.

الكلمات المفتاحية. زرع الخلايا الخيفية الجذعية الدموية ،الخلايا التائية الحاملة للمستقبل سي دي 4، التعافي، مرض الزراعة مقابل المضيف، مجموعات فرعية من الخلايا الساذجة والذاكرة، البقاء الكلي.