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*Original Article*

# Kinetics of Lymphocytes Reconstitution Post Allogeneic Hematopoietic Stem Cell Transplantation: Two years of Follow-up

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## ABSTRACT

***Background and aims.*** *Allogeneic hematopoietic stem cells transplantation (HSCT) is strong curative treatment for several classes of immunodeficiency, metabolic disorders, and haematopoietic malignancies. Depending on HSCT procedure, thousands of patients could heal from their underlying disease. The ability of hematopoietic stem cells transplantation (HSCT) to cure is affected by variant factors. The objectives of this study were to analyze the kinetics of lymphocyte recovery at different time points after allogeneic hematopoietic stem cells transplantation and to correlate their recovery with some factors that influence the transplantation clinical outcome.* ***Methods****. In this study, 16 consecutive allogeneic hematopoietic stem cell transplantation (HSCT) recipients were analysed during the first two-year post transplantation by measuring the absolute count of lymphocytes every three months for each patient. Data were presented as minimum, maximum and mean.* ***Results****. Frequencies of lymphocytes increased gradually during the first 24 months post HSCT and their recovery was affected by different factors such as graft source, patient age, and chronic graft versus host disease. Lymphocytes are essential for adaptive immune responses. Type of transplant, graft versus host disease, and recipient age affect their reconstitution.* ***Conclusion****. Proper lymphocyte recovery is associated with better clinical outcome and increased the survival rate.*

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## INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for patients with different malignant and non-malignant disorders; however, treatment efficacy is affected by by variant factors include: the kind of disorder [1,2], state of disease before the transplant [3], stem cell source[4], human leucocytes antigens (HLA)-matching compatibility between the donor and recipient [5,6], conditioning regiments [7,8], cell removal [9,10], graft versus host disease (GVHD) and its treatment [11], and post-transplant infections [12,13]. Immune reconstitution after allogeneic Hematopoietic stem cell transplantation (HSCT) generates from the donor-derived progenitors and the proliferation of the immune cells transferred with the graft.

There is a significant difference in the kinetics of innate and adaptive immune recovery and the rapid reconstitution of monocytes and natural killer (NK) cells 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 [14]. The Long defence insufficiency occurs mainly from deficiencies in effective cellular immune reconstitution [15-17].

Deficiency of B lymphocytes frequencies can be detected in few months post HSCT and influenced by chronic graft versus host disease (cGVHD) and / or its treatment [18-20]. Different kinds of procedures are performed to evaluate post-transplant immune recovery, including tests which done routinely in clinical laboratories (absolute lymphocytes counts, CD4+, CD8+, NK cells, B cells, and antibodies titers [21]. Recovery of lymphocytes from donor cells attack residual tumor cells early post transplantation and prevent relapse and thereby the absolute lymphocyte count serves as a predictor of the clinical outcome after HSCT [22]. Hence, this work was conducted to analyze kinetics of lymphocyte regeneration in 16 consecutive allogeneic hematopoietic stem cell transplantation recipients during two years of clinical follow-up, and

to compare their recovery according to different factors that affect transplantation outcome such as age of patient, chronic graft versus disease, and type of transplant.

## METHODS

### *Study design and setting*

An observational and descriptive study of patients undergoing allogeneic HSCT between January 2007 and January 2009 was carried out in laboratory of cellular therapy, Campus Virchow Clinic, Charite' University, Berlin, Germany. An ethical committee approval was taken from institutional ethical committee. Some patients received a myeloablative regimen, while others received a reduced –intensity regimen (depending on the underlying disorder) prior to allogeneic HSCT. All the recipients treated with Aciclovir as Antiviral prophylaxis and Anti Thymocytesglobulin, Cyclosporine-A, and Methotrexate as graft-versus –host disease (GVHD) prophylaxis. GVHD was defined as acute if it occurred before day 100 and chronic thereafter.

### *Data collection procedure*

The study cohort comprised 16 consecutive patients who underwent allogeneic hematopoietic stem cell transplantation (HSCT) with myeloablative or reduced-intensity conditioning. Patients receiving umbilical cord blood transplantation, or haplo-identical transplantation were excluded. Patients who died or relapsed were also excluded. The diagnosis they were referred to the hematopoietic stem cell transplantation unit for the following: Acute lymphoblastic leukemia, 38% (n=6); Acute myeloid Leukaemia, 25% (n=4); Wiscott-Aldrich syndrome, 13%% (n=2); Fanconi Anemia, 6% (n=1); Chronic myeloid Leukaemia. 6% (n=1); severe combined immune deficiency, 6 % (n=1); X-chromosomal Adrenoleukodystrophy, 6 %( n=1). About 10(63%) patients were males and 6(37%) were females; age ranged from 6 months to 22 years. Age of donors ranged from 7-50 years. Graft source was 69% (n=11) bone marrow and 31% (n=5) Peripheral Blood stem cell (PBSC). All patients received their graft from matched unrelated donors. 88% of the patients (n=14) had acute graft versus host disease, range from grade I –II, and 69% (n= 11) had grade II of chronic graft versus host disease.

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 Analyzer (Applied Biosystems, Inc., Foster City, CA). Alleles specific to donor or recipient were used for chimerism identification.

Fresh whole blood specimens were collected during the first two years post transplantation. Patient ʻs peripheral venous blood was collected into 10-ml Li-heparin/EDTA vacationer Becton Dickinson (BD, USA) after written informed consent. The study protocol was approved by laboratory of cellular therapy, Campus Virchow Clinic, Charité University, Berlin, Germany. The Absolute Lymphocyte counts at time point of the study were assessed from complete blood counts drawn as part of routine clinical investigation after transplantation. Absolute Lymphocyte count for every patient was quantified every three months during the first two years post-transplant.

### *Statistical analysis*

Statistics (means, minimal, and maximal values) were used to describe patient baseline characteristics. Results were presented as mean values of Lymphocytes, and p-values. Data was analyzed using SPSS version 18. Student's *t*-test was used to ascertain the significance of differences between mean values of two continuous variables and confirmed by nonparametric Mann-Whitney test.

## RESULTS

### *Reconstitution of Lymphocytes*

To analyze the lymphocytes reconstitution kinetics in all 16 patients, absolute count of lymphocytes/µl was measured in whole blood every three months during two years of follow- up. Counts of lymphocytes were measured and presented as mean, minimal, and maximal values. Mean of the lymphocytes increased from 1084/µl at first three months to 6998/ µl at 24 months.

***Table 1. Lymphocytes /µl during 24 months post allogeneic HSCT (Mean, Minimal-/ Maximal values)***

| Time post HSCT | Mean | Minimum | Maximum |
|---|---|---|---|
| **Three months** | 1084 | 333 | 1994 |
| **Six months** | 1669 | 799 | 3049 |
| **Nine months** | 2242 | 766 | 5416 |
| **Twelve months** | 2889 | 799 | 7488 |
| **Fifteen months** | 3568 | 865 | 7585 |
| **Eighteen months** | 5894 | 875 | 7693 |
| **Twenty-four months** | 6998 | 921 | 7733 |

***Impact of stem cell source on the Lymphocytes recovery:***

Upon the correlation with the factors that affect the regeneration of lymphocytes after allogeneic haematopoitic stem cell transplantation, we had compared the impact of graft source on the kinetics of lymphocytes recovey (Figure 1). The recovery of lymphocytes over time was significantly higher in patients having recieved bone marrow (BM) than in those transplanted from peripheral blood stem cells (PBSC) (P≤ 0.003).

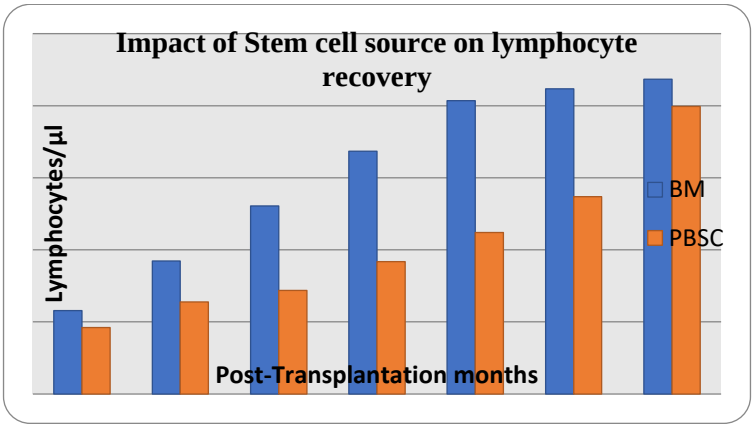


***Figure 1. Impact of graft source on lymphocyte recovery***

***Impact of chronic GVHD (cGVHD) on the recovery of Lymphocytes:***

The reconstitution of Lymphocytes over time was significantly higher in patients without chronic graft versus host disesae than in those with symptoms of chronic graft versus host (P= 0.007) (Figure 2).

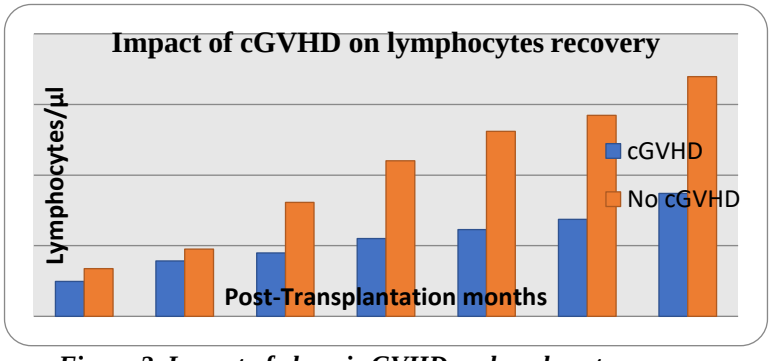


***Figure 2. Impact of chronic GVHD on lymphocyte recovery***

***Impact of recipient-age on the recovery of Lymphocytes***

The reconstitution of Lymphocytes over time was significantly higher in patients whoe aged < 15 years (P= 0.001). Their recovery was similar in both groups at 24 months (Figure 3).

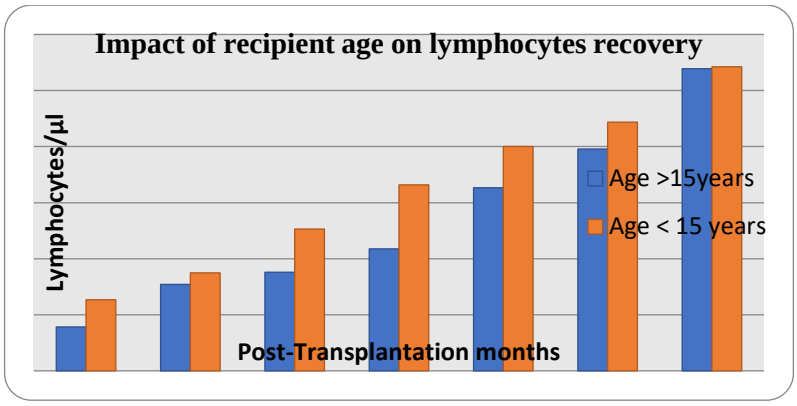


***Figure 3. Impact of patient age on lymphocyte recovery***

## DISCUSSION

Immune reconstitution has a major role in the clinical outcome after allogeneic hematopoietic stem cell transplantation (HSCT). The donor lymphoid reconstitution is a potent surrogate of immune recovery and the rapid lymphocyte regeneration is associated with a survival support post HSCT [23-25]. Allogeneic hematopoietic stem cell transplantation (HSCT) gives a unique chance to improve the evolution of immune regeneration. Potent immune development and defense against invading pathogens needs efficient lymphocytes recovery [26,27]. After HSCT, the major rules that are very serious for a successful transplantation include engraftment of the transplanted stem cells; inhibit graft versus host disease (GVHD) and regeneration of active lymphocytes [28].

Rapid lymphocytes regeneration post HSCT is necessary for the reaching these goals. The lymphocytes regeneration and maturation from hematopoietic stem cells occurs in the thymus and bone marrow. In their tissues, lymphoid progenitor cells rapidly proliferate and differentiate to mature cells with highly diverse receptor repertoire. These Naive cells can react with different types of antigens [29-31]. Lateness of immune recovery post HSCT was accompanied with high rate of infections and relapse [32-34]. Immune responses which mediated by lymphocytes is influenced by different factors include conditioning treatment, thymic impairment in the host, graft source, graft dose, T-cell depletion, donor-host disparity, acute and chronic graft-versus-host disease (GVHD) preventive treatment or therapy.

Different studies have focused on evaluating Lymphocyte frequencies post HSCT, as they play a major role in the improvement of the clinical outcome after HSCT. In this study, we investigated kinetics of lymphocyte recovery post allogeneic HSCT and evaluated the impact of different factors on their recovery. We have found that the reconstitution of lymphocytes increased gradually during the first two year after allogeneic HSCT and related with long-term survival. The impact of the graft source on lymphocyte regeneration has been previously described and bone marrow as the source of allograft was associated with a higher lymphocyte's recovery [35]. Absolute count of lymphocytes was significantly higher in patients without the occurrence of later episode of chronic GVHD. Furthermore, early lymphocytes recovery was better in the patients who aged < 15 years espcially in the first eighteen months with low rate of infections and relapse [36].

The current study has evaluated matched unrelated HSCT and observed that higher lymphocyte counts were associated with better outcome, because of a higher survival rate, lower mortality rate, less relapse, and less severity of graft versus host disease (GVHD). Faster lymphocyte reconstitution after HSCT may be a sign of more rapid immune recovery, leading to fewer infections and deaths. Low post-transplantation lymphocyte counts have been associated with cytomegalovirus (CMV) infection as well as with elevated rate of death from CMV disease. [37].

## CONCLUSION

The finding of this study evaluated kinetics of lymphocyte reconstitutions during two years after HSCT and suggested that the absolute lymphocyte count (ALC) early after HSCT may have significant prognostic values. Low ALC early post HSCT is associated with higher risk of non-relapse mortality and poor survival because of graft versus host disease and

infections. Furthermore, type of transplant, patient age, and chronic graft versus host disease have a significant impact on lymphocyte reconstitution at different time points after allogeneic HSCT.

***Disclaimer***

The article has not been previously presented or published, and is not part of a thesis project.

***Conflict of Interest***

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

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