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Recovery of Regulatory T cells after allogeneic hematopoietic stem cell Transplantation

Eida Elmansorry
Department of medical laboratories, faculty of medical technology, Tripoli University, Libya

Abstract:
Background*: Regulatory T cells play a critical role in maintenance immunological tolerance while preserving tumour and microbial immunity. Several clinical trials have found that Tregs can treat and prevent graft versus host disease (GVHD, without increasing the risk of relapse and infection. Tregs are identified by co-expression of CD4, high expression of CD25 and Foxp3. Foxp3is specifically expressed in Tregs and is important to control their stability and development. . **Materials and Methods**: In this study, 20 consecutive s allogeneic hematopoietic stem cell transplantation (HSCT) recipients during the first year post transplantation were analysed by measuring the percentages of regulatory T cells every month for each patient. Data were presented as minimum, maximum and mean. **Results**: Tregs increased gradually during the first 12 months post HSCT and their recovery was affected by different factors such as graft source, underlying disease, and chronic graft versus host disease. **Conclusion:** Regulatory T lymphocytes (Tregs) are essential for peripheral immune tolerance. Type of transplant, graft versus host disease, and original disease affect their reconstitution.*

Key Word: *Allogeneic Hematopoietic stem cell transplantation, Regulatory T cells, Reconstitution, graft versus host disease, Recovery*

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I. 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 the complications of graft versus host disease (GVHD) and infections.

Regulatory T cells (Tregs) are subset of $CD4^+$ T cells which have a suppressor function as they help through regulating immunologic self-tolerance while preserving tumor and microbial immunity. Different studies have worked for the characterization of allospecific regulatory T cells and evaluation of their importance for the therapy of autoimmune diseases and transplantation complications. [1,2,3]

A transcription factor called Foxp3, a member of the fork head family of transcription factors, is important for the stability and development of Tregs and is used as identifying marker for Tregs. [4,5] Tregs are mature subset of T cells and can be also induced from $CD4^+CD45RA^+$ naïve T cells in the periphery. [6] Natural Tregs are produced from the thymus and are identified by co-expression of CD4, high expression of CD25 and Foxp3. [7]

Tregs play a key role in transplantation tolerance in experimental models of skin and /or solid organ transplantation [8] as well as tolerance to allogeneic bone marrow transplantation. [9] recent studies have shown that $CD4^+$ $CD25^+$ Tregs were able to suppress GVHD after bone marrow transplantation while preserving graft versus leukemia or graft-versus-tumor effect. [10]

II. Material And Methods

This observational and prospective study of patients undergoing allogeneic HSCT between March 2008 and March 20009 was carried out in laboratory of cellular therapy, Campus Virchow Clinic, Charite' University, Berlin, Germany. A total of twenty consecutive patients (both male and females) of aged between 0.5- 26 years were included in this study.

Study Design: Prospective observational study
Study Location: This study was carried out in laboratory of cellular therapy, Campus Virchow Clinic, Charite' University, Berlin, Germany.

Study Duration: March 2008 to March 2009.
Sample size: 20 patients.

Sample size calculation: The sample size was estimated on the basis of a single proportion design. The target population from which we selected our sample was considered 35. We excluded the patients who died at the begging of the study and the patients who relapsed were also excluded.

Subjects & selection method: The study population was drawn from consecutive patients who undergoing allogeneic HSCT presented to Hematopoietic Stem Cell Transplantation Unit **,**Campus Virchow Clinic, Charite' University, Berlin, Germany between March 2008 to March 2009.

Inclusion criteria:

1. Patients who transplanted between March 2008 to March 2009
2. Aged ≤26 years,
3. Patients who transplanted from matched unrelated donor

Exclusion criteria:

1. Patients who died in the first three months after HSCT
2. Patients transplanted for sickle cell disease
3. Patients who relapsed were excluded at the date of relapse.
4. Patients with limited number of analysis at determined time point were also excluded.

Procedure methodology

After written informed consent was obtained, Fresh whole blood specimens were collected once on day 30, day 60, day 90, day 120, day 150, day 180, day 210, day 240, day 270, day 300, day 330, and day 360 post transplantation. Patient's peripheral venous blood was collected into 10-ml Li-heparin/EDTA vacationer Becton Dickinson (BD, USA) after informed consent .The study protocol was approved by laboratory of cellular therapy, Campus Virchow Clinic, Charite' University, Berlin, Germany.

Patients and transplant characteristics are presented in Table 1. All patients received Cyclosporine A as GVHD prophylaxis, with either mycophenolate mofetil or Methotrexate. GVHD was defined as acute if it occurred before day 100 and chronic thereafter.

Lymphocyte were analyzed using four-color FACS CAN (BD, USA) flow cytometer Tregs were detected using Allophycocyanin (APC)-conjugated anti-CD4(clone SK3), and Phycoerythrin(PE)-conjugated anti-CD25(clone 2A3), as this phenotype has been correlated with FOXP3 intracellular expression.

Aliquots of 50 microliter of EDTA/ Heparin blood were placed in FACS tubes (BD, USA) and stained with the appropriate antibodies (titrated for optimal concentration) ,then incubated shortly in the dark place. Finally the erythrocytes were lysed, washed, and FACS-lysing solution, (BD Pharmingen, USA) was added for the final fixation. Cells were analyzed on FACS CAN (BD, USA) flow cytometer .Data was further analyzed using Cell Quest program software. Tregs for every patient were gated and quantified every month from day 30 until day 360 post transplant. Regulatory T cells were presented as a percentage of total CD4+ T cells.

Statistical analysis

Statistics (means, minimal, and maximal values) were used to describe patient baseline characteristics. Results are presented as mean values of Treg percentages, 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.

III. Result

Table no 1: Shows Patients and transplant characteristics

	Number (%)
Males **Females**	12(60%) 8(40%)
Age (mean, min-max)	10.8(0.5-26)
Donor age (mean, min-max)	30(7-50)
Stem cell source Peripheral blood Bone marrow	 5(25%) 15(75%)
Acute GVHD (grade I-II) **Chronic GVHD**	18(86%) 15(75%)
Hematological disease Acute lymphoblastic leukaemia Myelodysplastic Syndrome	 7(35%) 3(15%)

Acute myeloid Leukaemia	2(10%)
Wiscott-Aldrich syndrome	3(15%)
Fanconi Anemia	2(10%)
Chronic myeloid Leukaemia	1(5%)
severe combined immune deficiency	1(5%)
X-chromosomal Adrenoleukodystrophy	1(5%)

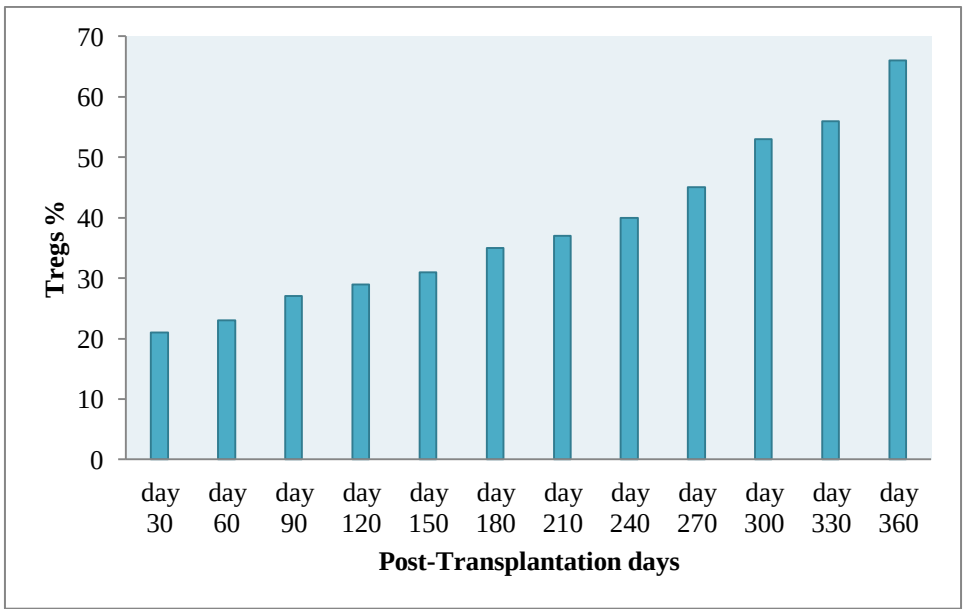
Reconstitution of Regulatory T cells (Tregs):

To analyze Treg reconstitution kinetics in all 20 patients, frequencies of Tregs cells expressing $CD4^+$ and $CD25^{hi}$ surface markers were measured in whole blood from day 30, day 60, day 90, day 120, day 150, day 180, day 210, day 240, day 270, day 300, day 330, and day 360 post HSCT . Percentages of $CD4^+$ $CD25^+$ cells were measured and presented as mean, minimal, and maximal values.

Mean of the Tregs increased from 21% of total $CD4^+$T cells at first month to 66% at 12 months, but always remained low compared to healthy controls. The increase in Treg percentage of total $CD4^+$ T cells over time was statically significant ($P \leq 0.002$) from day 30 to day 365 after allogeneic HSCT.

Table no 2: Tregs in different time points post allogeneic HSCT (Mean, Minimal-/ Maximal values)

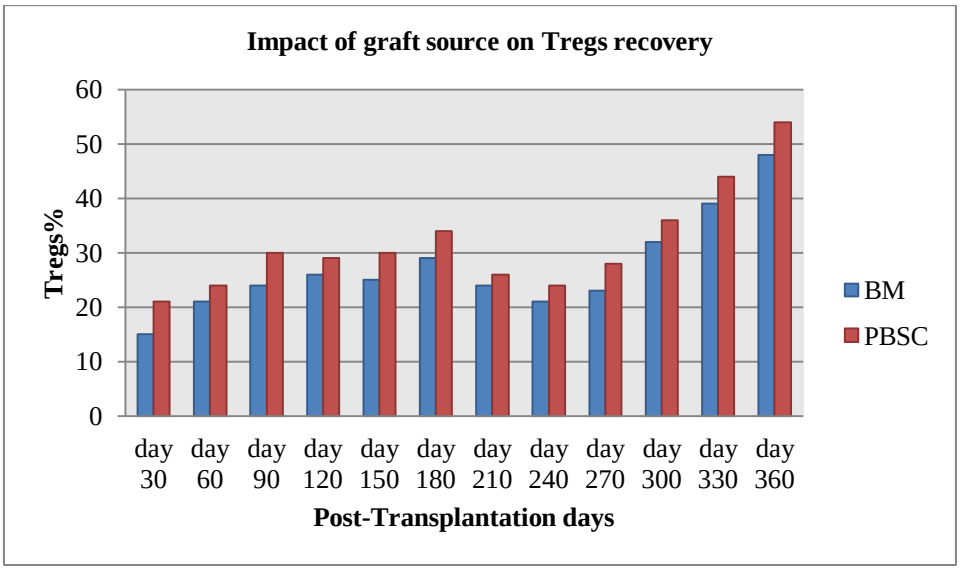
Time post HSCT	Mean of Tregs %	Minimum	Maximum
Day 30	21	3	45
Day 60	23	4	50
Day 90	27	8	57
Day 120	29	9	58
Day 150	31	9	59
Day 180	35	10	67
Day 210	37	10	69
Day 240	40	11	70
Day 270	45	12	71
Day 300	53	12	72
Day 330	56	15	73
Day 360	66	22	78



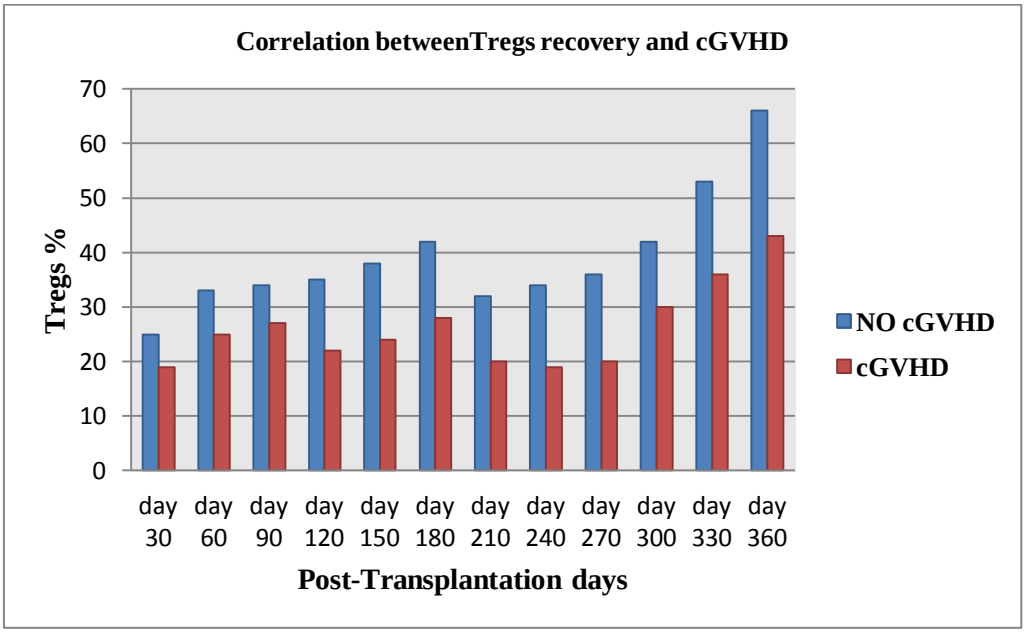
Impact of graft source on the recovery of Tregs:

Upon the correlation with the factors that affect the regeneration of Tregs after allogeneic haematopoitic stem cell transplantation, we had compared the impact of graft source on the kinetics of Tregs recovey.The recovery

of Tregs over time was significantly higher in patients having recieved peripheral blood stem cells (PBSC) than in those transplanted from bone marrow (BM) (P≤ 0.001) .



Impact of chronic GVHD (cGVHD) on the recovery of Tregs:
The reconstitution of Tregs over time was significantly higher in patients without chronic graft versus host disesae) than in those with symptoms of chronic graft versus host (P= 0.009).



Impact of underlying disease on the Tregs recovery
At six months post- allogeneic HSCT, Treg reconstitution was significantly higher in Patients transplanted for malignant diseases reconstitution than patients transplanted for nonmalignant disorders (p≤0.03). After 12 months post allogeneic HSCT, Treg reconstitution was significantly higher in Patients transplanted for nonmalignant disorders (p≤0.02).

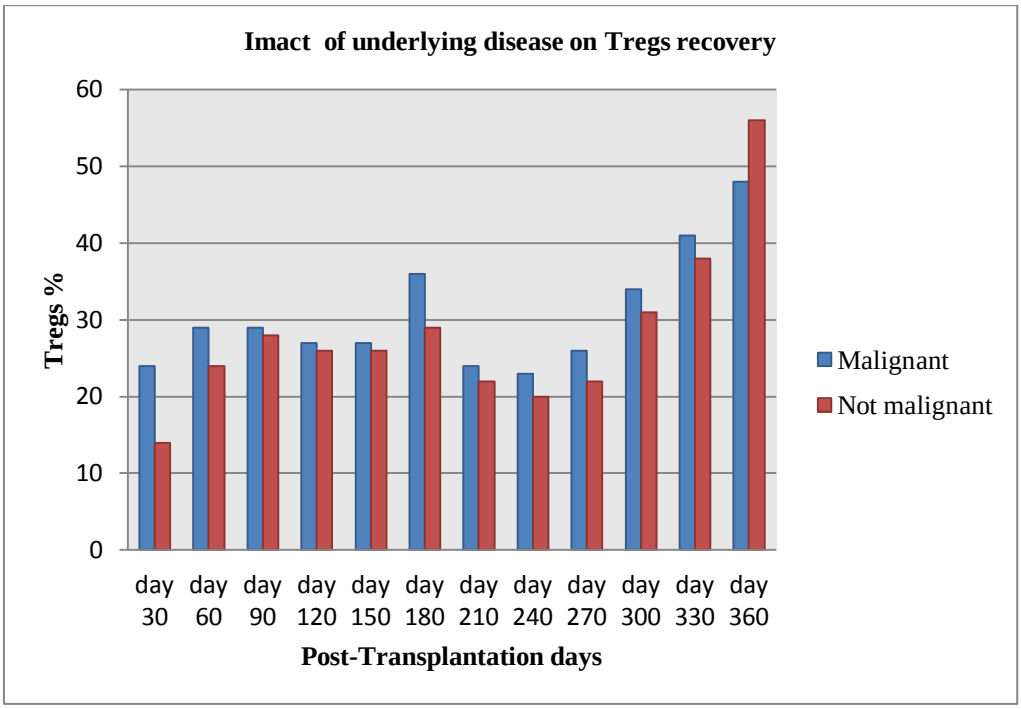


Table no 3: Shows Factors influencing Treg recovery

Treg recovery	P value
Graft source: PBSC versus BM	≤ 0.001
Chronic GVHD: no versus yes	0.009
Underlying disease: Malignant versus non-malignant before day 360	≤0.03
Underlying disease: Malignant versus non-malignant after day 360	≤0.02

IV. Discussion

Regulatory T cells play a critical role in both solid organ transplant tolerance and in allogeneic transplantation tolerance. Tregs have strong suppressive activity. They are population of thymus-derived naïve $CD4^+$ T cells that co-express the Interleukin -2 R alpha chain, CD25. [11]

Different studies have focused on evaluating Tregs frequencies post HSCT, as they play a major role in the improvement of graft versus host disease. In this study, we investigated Treg regeneration post allogeneic HSCT and evaluated the impact of different factors on their recovery. We found that the reconstitution of Treg increased during the first year after allogeneic HSCT. Peripheral blood stem cell (PBSC) as the source of allograft was associated with a better Treg recovery. Frequencies of Treg relative to CD4+ T cells were significantly higher in patients without the occurrence of later episode of chronic GVHD. Furthermore, patients transplanted for malignant diseases had a better Treg recovery during the first year post allogeneic haematopoietic stem cell transplantation.

The impact of the graft source on Treg regeneration has been previously described. Two studies, reported higher Treg recovery at 6 months in patients transplanted from PBSC than after cord blood transplantation.[12, 13]

Different studies found that the regulatory T cells effectively decreased the incidence and severity of graft versus host disease. [10, 14, 15] other studies identified regulatory T cells based on PCR analysis of Foxp3 expression found that the patients with GVHD had smaller numbers of Tregs. [16, 17]

Our study showed that the patients who transplanted for malignant diseases had a better Treg regeneration during the first year after HSCT. These finding confirmed the role of Tregs in the treatment of malignant haematological diseases by destroying remaining tumor cells and prevent relapse after transplantation. [10, 14]

V. Conclusion

Many studies have described the importance role of T-cell in regulation of immune responses and provide critical information about their function of balancing immunologic self-tolerance while preserving tumour and microbial immunity. Regulatory T cells are defined as a key cellular component that mediates this

process. The identification of cell surface markers and intracellular molecules has led to further characterization of their populations. Regulatory T cells have a potential role in the regulation of self-tolerance, autoimmunity, tumour and microbial immunity, and alloresponses in solid organ and hematopoietic cell transplantation (HSCT). This study has described the kinetics of Treg recovery after allogeneic haematopoietic stem cell transplantation and described different factors influencing their regeneration such as graft versus host disease and graft source.

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