Immune and Hematological Reconstitution after Allogenic Bone Marrow Transplantation in Tunisian Pediatric Recipients: Prospective Study and Tunisian Experience Report

F. Jenhani[1,3,*], Z. Regaya[1], L. Berraies[1] and F. Mellouli[2]

[1]*Cellular Immunology and Cytometry and Cellular Therapy Laboratory, National Blood, Transfusion Center, Tunis-Tunisia, 13 Rue Djebel Lakdar, Bab Saâdoun 1006, Tunis, Tunisia*

[2]*Bone Marrow Graft National Center, Tunis-Tunisia, 13 Rue Djebel Lakdar, Bab Saâdoun 1006, Tunis, Tunisia*

[3]*Immunology Research Unit, Faculty of Pharmacy of Monastir, University of Monastir Avenue Avicenne Monastir 5000, Tunisia*

Abstract: *AIM*: A regular monitoring of the immune reconstitution mainly based on the quantitative determination of lymphocyte T subpopulation. This is prospective analysis for 1 year in Tunisian children treated with allogenic intrafamilial bone marrow transplantation.

Methods: We conducted a prospective analysis for 1 year follow up enrolling 25 children treated with allogenic intrafamilial bone marrow transplantation among them two cases of Peripheral hematopoietic transplantation and placental cord blood transplantation including: aplastic anemia (6 cases), hemoglobinopathies (12 cases), myelodysplastic syndrome (1 case), 2 cases of Acute lymphocytic leukemia, a case of congenital amegacarycytosis and 3 cases of primary immunodeficiency with lack of expression of major MHC class II. All subjects received different conditioning regimens according to the indication. Our study consisted of a regular monitoring of the immune reconstitution mainly based on the quantitative determination of lymphocyte T subpopulation. So, these tests were routinely requested to 1 month, 2 months, 3 months, 6 months, 9 months and 12 months post- bone marrow transplantation.

Results: The average time of engraftment was 18 days corresponding to neutrophil recovery (12-24). For the T cell recovery, a rate of CD4 + T lymphocytes > 200/ mm3 was provided within an average of 2.5 months (1-7). The average time to obtain CD8+ T lymphocytes >200 /mm3 was 2 months (1-5). The humoral immune reconstitution was made within an average of 2 months (1-4). A ratio of CD4+ / CD8+ T lymphocytes (>1) was obtained within 10 months and a half (1-24). Univaried analysis showed a significant correlation between the bone marrow sex matched and the faster reorganization of CD8 + T cells (p = 0.042). Moreover, a quantity of CD34 +> 6x 10^6/ kg was significantly associated with the recapture of a formula lymphocyte T CD4+ / CD8+ (> 1) (p=0.03).

Conclusion: The immune recovery post bone marrow transplantation in children began with myeloid lineage then lymphoid B then lymphoid T. The inversion of the ratio CD4 +/CD8+ T lymphocytes, seemed to be influenced on the one hand by the high content of CD34 + cells in the graft as well as the type of conditioning on the other hand by the CMV infection since it accelerates significantly CD8+ T lymphocyte reconstitution.

Keywords: Immune reconstitution, monitoring, lymphocyte T cell enumeration, CD4+/CD8+ ratio, CMV infection, bone marrow transplantation, pediatric tunisian patients.

1. INTRODUCTION

Allogenic bone marrow transplantation (allo-BMT) is a possibly curative treatment of otherwise incurable malignant hematologic disorders and severe immunodeficiency diseases. After allo-BMT, the recipients demonstrate a varying period of immune incompetence that can last for up to several years after transplantation and may cause significant morbidity and mortality [1]. Immune reconstitution after allo-BMT has been studied extensively in adults [2,3,4] and thought to be dependent upon the ability of the hematopoietic graft to generate lymphoid and myeloid lineage cells *de novo* and on the function of mature cells contained in the graft. Post-transplantation, the different WBC populations regenerate at different tempos. The first cells to reconstitute are those of the innate immune response, including granulocytes, monocytes, macrophages and NK cells [5, 6]. Because the quantitative return of these cells correlates with return of significant protective immunity against bacterial pathogens, it has been assumed that these cells function normally. In contrast, the levels of T and B lymphocytes remain markedly reduced and function abnormally for several months or years post-transplantation. Attention has been focused primarily on T cells since many aspects of T cell function, including T cell helper, T cell induced inflammation and T cell cytotoxicity have been observed to be significantly impaired in the post-HCT patient [75].

*Address correspondence to this author at the Cellular Immunology, Cytometry and Cellular Therapy Laboratory, National Blood Transfusion Center BP 294 EL MANAR II 2092, Tunisia; Tel: + 21698817308; Fax: +21698724925;
E-mail: faouzi.jenhani@yahoo.fr, tunisiacelltherapy@ymail.com

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So as we already know that CD8 cell counts recover rapidly (by 3 months post-transplant) and are often normal or supranormal at 1 year, however CD4 T-cell numbers recover slowly with subsequent reversal of the normal CD4:CD8 ratio [7]. So, CD4+ T-cell counts kinetics vary with age and transplantation tempo and are <200 cells/ml during the first 3 months after bone-marrow transplant and slowly rise to 300 cells/ml by 1 year and 450 cells/ml at 5 years; then become normal 20-30 years post transplant [8,9,10]. In children, T cell recovery tempo seemed to be faster. So, in this study we tried to focus on the average time of the reconstitution of myeloid and lymphoid lineages and identify factors that may influence this organization.

2. MATERIALS AND METHODS

2.1. Patients and Therapy

We enrolled in this study 25 pediatric patients (16 boys and 9 girls) who were candidates for allogenic stem cell transplantation among them two patients received peripheral blood stem cells and a placental cord blood as graft and their corresponding donors at the National Bone Marrow Transplantation Center of Tunis-Tunisia. Transplantation was performed in an HLA geno- identical situation with 23 related and only two unrelated patients.

Table 1: Patients and Donors' Characteristics

	Parameters	***Grouping***	***Total (N=25)***
Recipients (Patients)	Age (median= 4 ans) range (0,7-12) years	<=2 years	8
		[2,4] years	6
		[4,6] years	4
		[6, 8] years	4
		>8 years	3
	Sex	Female	9
		Male	16
	Pathology	major β- Thalessemia	10
		aplastic anemia	6
		CID: combined immune deficiency with HLA II deficiency	3
		Acute lymphocytic leukemia	2
		sickle cell disease	2
		Myelodiysplastic syndrome	1
		congenital macrocytosis	1
Transplantation	GVHD prophylaxis	cyclosporine	4
		Ciclosporine and méthotrexate	19
		Ciclosporine and corticosteroid	2
	HSC sample	Bone marrow	23
		Peripheral stem cells	1
		Placental cord blood	1
	MNC (mononuclear cells) (10e8/kg)	<= 4	13
		[4,8]	11
	CD34+ (10e6/kg)	<=4	6
		[4,8]	13
		>8	6
Donor	Age (Median=12 years)	range (4-41)	25
	Sex	Female	10
		Male	15
	Origin	Related	23
		Unrelated	2
Donor /Patient	Sex-matching	Sex- matched	10
		mismatched	15
	ABO Incompatibility	Minor	6
		Major	4
		absent	14
	HLA Compatibility	geno-identical	25
	CMV serology	D+/R+	6
		D+/R-	3
		D-/R+	2
		D-/R-	11

The median age of patients was 4 years (7 months -12 years), and was 12 years for the donors (4 years -41 years).

All patients' parents gave written informed consent, which was approved by using the protocols approved by our hospital's Ethical Committee. Patient and donor characteristics are shown in Table **1**.

Donors were excluded if they were positive for HIV antibody or hepatitis B surface antigen or otherwise did not meet the transplantation criteria of the BMT center.

2.2. Patients' Diagnoses Include

6 cases of very severe aplastic anemia. The severity criteria were defined by the criteria of Camitta 1. A case of myelodysplastic syndrome, 2 cases of Homozygous sickle cell disease complicated to acute chest syndrome, 10 cases of major beta thalassemia, 2 cases of Acute lymphocytic leukemia, a case of congenital amegacaryocytosis and 3 cases of primary immunodeficiency with lack of expression of major MHC class II.

All patients included in our study didn't meet such exclusion criteria as the occurrence of death before exit from aplasia. While in the hospital, patients received prophylactic measures following isolation in a laminar flow chamber from the beginning of conditioning until neutropenia exit, digestive antibacterial decontamination by a local antibiotic action by gentamicin + collimycin, sterile supply and care mouth.

Post transplantation antifungal prophylaxis with fluconazole from day-11 till 3 months after transplantation. All patients received a dose of 25mg/kg/48h of cotrimoxazole as Pneumocystis carinii Prophylaxis starting from neutrophil recovery until 1 year after BMT.

Patients at risk for CMV disease underwent prophylactic therapy by Zovirax at a dose of 500mg/m^2 as 3 doses daily from day -5 until day +90. Complications related to ABO incompatibility were prevented by the graft deserythrocytation in cases of major or mixed ABO incompatibility in 4 patients and deplasmatisation in cases of minor ABO incompatibility in 6 patients.

Conditioning chemotherapy was administered according to the patient's actual or ideal body weight (Table **1**). Patients with haemoglobinopathies (13 patients) received Busilvex IV (equivalent of busulfan as total dose of 16mg/kg from day-10 till day-7) followed by cyclophosphamide (a total dose of 200mg/kg from day-5 till day-2) then antilymphocyte serum at a dose of 15mg/kg from day-4 till day-2. While for patients with a defect of expression of MHC class II (3 patients), conditioning chemotherapy consisted only of busulfan (16mg/kg from day-10 till day-7) and Cyclophosphamide (200mg/kg from day-5 till day-2). Concerning one patient who required a second transplantation, he received Busilvex IV at a dose of 8 mg/kg from day-10 till day-7 followed by a dose of 40mg/m^2 of Fludarabine daily for 4 days and a total dose of 1mg/kg of Compath. Patients with severe aplastic and acquired anemia (3 children) received a total dose of 200mg/kg of Cyclophosphamide followed by a total dose 90mg/kg of ATGAM (anti lymphocyte serum (ALS)) starting from day-5. A child with myelodysplastic syndrome had received busulfan (16mg/kg) followed by Cyclophosphamide (200mg/kg). 2 cases with acute lymphoblastic leukemia have had a chimiotherapy consisting of a total body irradiation during 3 days then a total dose of 200mg/kg of Endoxan. Finally, a child with Fanconi anemia had received a lesser dose of Fladarabine (total dose of 25mg/m^2SC) followed by a little dose of Cyclophosphamide (total dose of 80mg/kg) starting from day-5for day-2.

All patients received cyclosporine and methotrexate as GVHD prophylaxis. Cyclosporine IV as a total dose of 3 mg/kg from day-1 till neutropenia exit subsequently relayed by 6-8mg/ kg/day orally in order to reach a residual rate of ciclosporinemia between 150 and 250ng/ml. Methotrexate was administered intravenously on day+1 (15mg/m^2), then at 10mg/m^2on day+3, and+6 in 16 patients only.

All 23 patients received a BMT graft in genoidentical situation containing a median of mononuclear cells (MNC) of 5 x10^8 /kg (range 2.1x 10^8-2.9.8x10^8) and a median CD34+ cells dose of 7.46x10^6/kg (1.85x10^6-21.03x10^6), with a median of viability of 93.84% (range 83.68%-98.37%).

2.3. Laboratory Analysis

In this study, neutropenia exit was defined by a neutrophil count (ANC) (>500/mm^3) on at least two consecutive days. The study of the immune reconstitution kinetics has been realized by flow cytometry based on monitoring T cell sub populations only.

So, blood samples from each patient were analyzed by direct flow cytometry regularly on 1, 2, 3, 6 and 12

months after allogeneic bone marrow transplantation to assess the immunological reconstitution. We used Human monoclonal antibodies (mAbs) (CD3, CD4, CD8, CD45) and isotype controls (Becton Dickinson, San Jose´, CA, USA). Briefly, appropriate amounts of mAbs were added to 100 µl of whole blood followed by an incubation of 15 min at room temperature. Red cells were then lysed and then preceded to enumeration of T lymphocyte subpopulation T CD3+, CD4+, CD8+ by flow cytometer (BD FACScalibur) and analyzed by Cell Quest software.

T cell reconstitution was defined by a rate of CD4 (> 200/mm^3) and CD8 + T (cells> 200/mm^3).

Complete blood counts with differentials were performed regularly on 1 month, 2 months, 3 months, 6 months and 12 months after BMT and were made by means of an automated Beckman Coulter HMX.

Data corresponding to the factors that may influence the immune restoration were collected and concern particularly donor age, recipient age and gender, their corresponding blood groups, the quantity of CD34 + cells content in the graft, type of conditioning chemotherapy used, presence of antibodies anti-HLA before transplantation, the presence of complications post graft like infection or GVHD.

The diagnosis of GVHD was confirmed by histology. The degree of severity was measured by grade of Glucksberg for acute GVHD [11].

2.4. Statistical Analysis

For statistical analysis, SPSS for Windows was used for the independent samples. Data from our study were entered on SPSS 17. Comparisons averages were made by the Student *t* test and the applicability of this test is already checked. Otherwise, we used nonparametric tests, such as Wilcoxon test. Comparisons percentages were made by the Pearson chi-square test or Fisher's exact test when appropriate. P(<0.05) was required for statistical significance.

3. RESULTS

The median time to neutrophil recovery was 18 days ± 4 with a median of 4x10^3/µl ± 3x10^3 Neutrophil engraftment was more rapid in patients who didn't receive Methotrexate for GVHD prophylaxis occurring at (14 days ± 3 vs 18.6 days ± 4, *P*=0.04) than that in subjects who received this molecule.

Concerning T cell distribution, a rate of CD4 + T lymphocytes (>200/mm^3) was obtained within a median of 3 months. The average time of T cells CD8 (>200/mm^3) was about 3 months. So, T cell population monitoring was regularly evaluated each 3 months till +1 year post transplantation. Total lymphocyte count grew up continuously from +1 month till +12 months post allograft and reached a normal value (>1x10^3/µl) at 90 days. However, considering all patients, we do not find significant differences according to T lymphocyte CD3+ at 3 months, 6, 9 then 12 months following transplantation (Figure **1**).

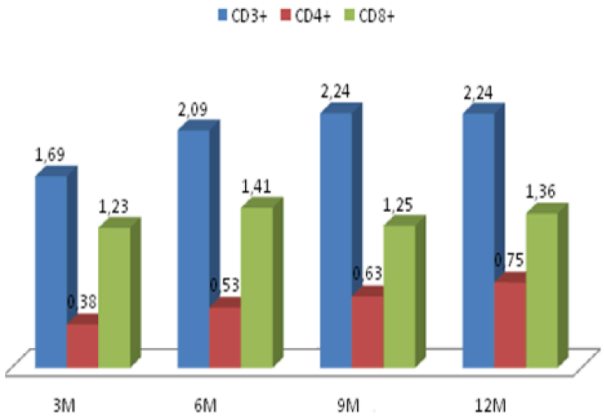

Figure 1: Circulating CD3+, CD4+ and CD8+ T cells counts from 3 months till 1 year after allogenic hematopoietic stem cell transplantation.

3.1. Factors Influencing T Cell Reconstitution

3.1.1. Impact of Recipient's Age

In the present study we found that recipient age (Figure **3**) appeared to have an impact on the T cell recovery; so more the patient is younger, whose CD4+ T cell being replenished quickly. While, CD8+ T cells counts appear to be less adversely affected by this factor.

A significant difference is revealed by comparing CD3+ T cell counts of the younger patients (2 years) with those of older ones mainly at 6 months (> 3x10^3 TCD3+/ µl vs <2.4x10^3 TCD3+/ µl, p = 0.02) and at 9 months (> 3.5x10^3 TCD3+/ µl vs <2.5x10^3 TCD3+/ µl, p = 0.03) after HSCT. Similar results were found when considering CD8+ T cells counts (>2.5 x10^3 TCD8+/ µl vs < 1.7x10^3 TCD8+/ µl p=0.05 at 6 months) and (>2x10^3 TCD8+/ µl vs < 1.5x10^3 TCD8+/ µl, p=0.05 at 9 months). Whereas the rate of CD4+ T cells reconstitution was lower, reached its maximum rate of 10^3/µl in all patients with no significant differences noted (Figure **2**).

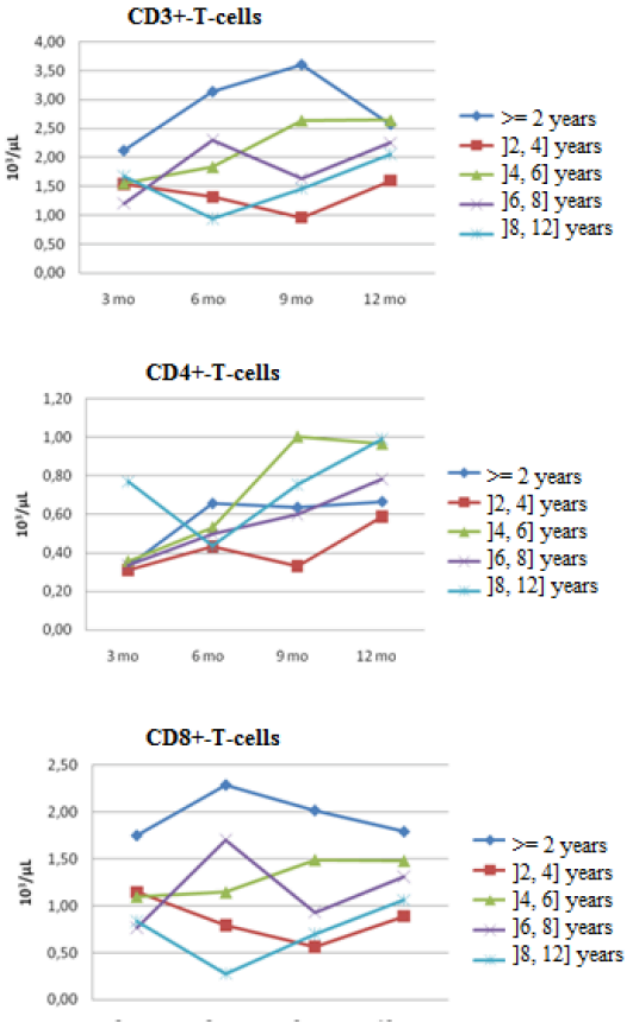

Figure 2: Circulating T cells subpopulations counts (CD3+, CD4+ and CD8+) considering the recipient's age, throughout 1 year post allogenic HSC transplantation.

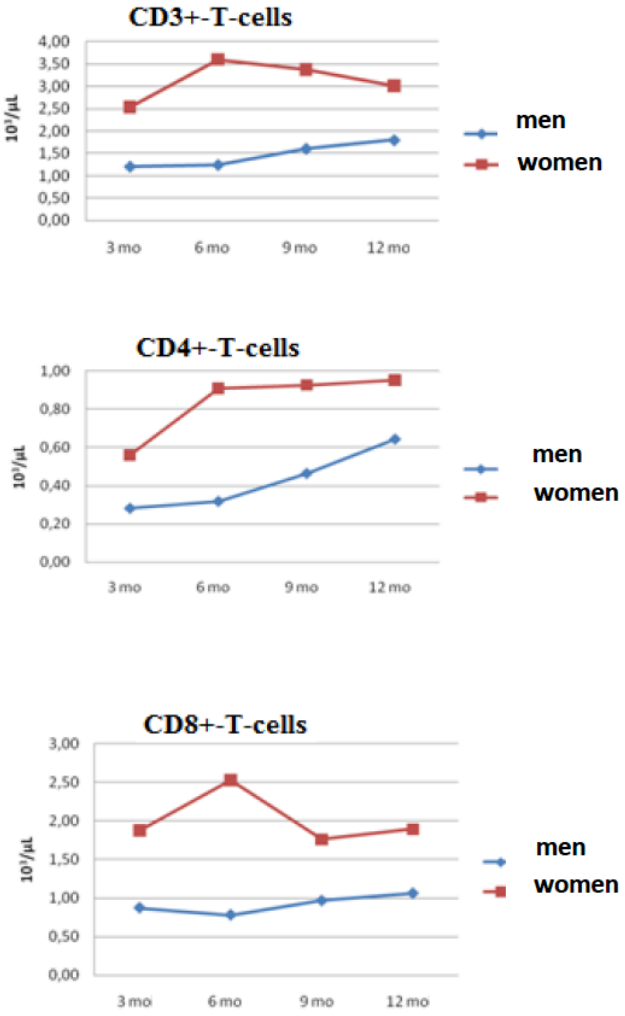

Figure 3: Circulating T cells subpopulations counts (CD3+, CD4+ and CD8+) considering the recipient's sex, throughout 1 year post allogenic HSC transplantation.

3.1.2. Impact of Recipient's Sex

Focusing on our study population we found out that recipient's sex affected also T cell reconstitution. Especially CD3+ subpopulation recovery was clearly important in girls than in boys ($1.21x10^3$TCD3+/µl for boys vs $2x54.10^3$TCD3+/µl for girls, P=0.05 at 3 months after HSCT). Moreover TCD4+ ($0.9x10^3$ TCD4+/µl for boys vs $0.61x10^3$ TCD4+/µl for girls, p< 0.05) and TCD8+ ($1.9x10^3$ TCD8+/µl for boys vs $1.19x10^3$ TCD8+ /µl for girls, p<0.005) cells reconstitutions revealed also variable distribution between the 2 sexes reaching statistical significance throughout 1 year post-HSCT (Figure **3**).

3.1.3. Impact of Pathology

Immune reconstitution is evident to depend mainly on recipient's disease. In our study population we observed an important variability between patients concerning the whole T cells subpopulations. So higher significant T(CD3+: $2.5x10^3$ cells/µl vs $1.1x10^3$ cells/µl, p<0.01 and CD4+: $0.78x10^3$ cells/µl vs $0.3x10^3$ cells/µl, p<0.01 and CD8+: $1.7x10^3$ cells/µl vs $0.5x10^3$cells/µl, p<0.01)cells recovery was observed in patients with major β - Thalessemia compared to those with sickle cell disease. The latter had the lower rates (Figure **4**).

3.1.4. Impact of CMV Serology

CMV infection is of major concern to the transplanted patients and immune response to CMV infection is mainly cellular cytotoxic. So after stem cell transplantation patients usually face different risks of CMV infection according to the serological status of both donor and recipient. If the couple donor/recipient is double negative (D-/R-) which was represented by 11 cases in our study population, the risk is considered

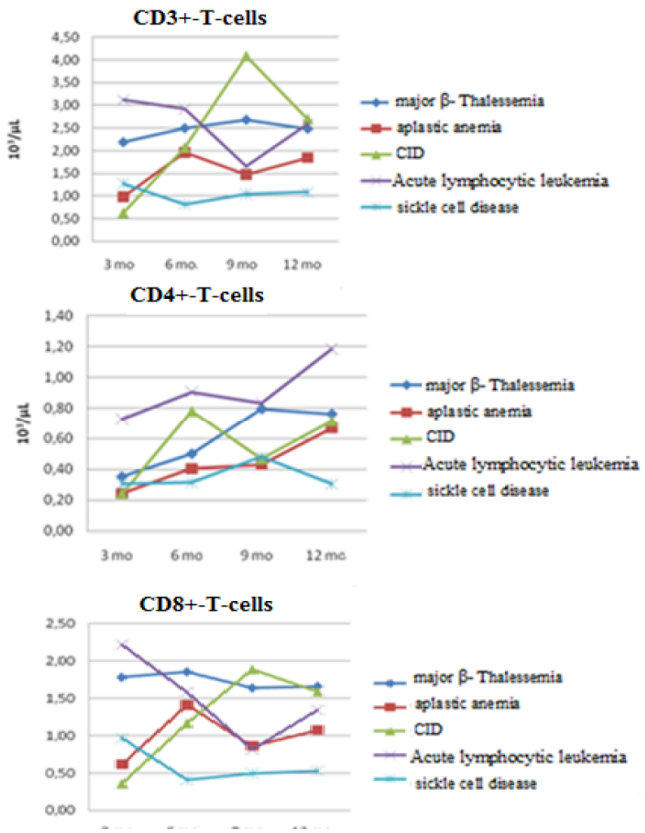

Figure 4: Circulating T cells subpopulations counts (CD3+, CD4+ and CD8+) regarding the recipient's underlying pathology, throughout 1 year post allogenic HSC transplantation.

low. However, the risk is considered higher among donor/recipient where only the recipient is seropositive (D-/ R+), and considered as an intermediate risk for couples with a seropositive donor (D+/R+ and D+/R-).

In our study population, only 2 patients hold a high risk for CMV infection and 9 patients with an intermediate risk.

Whole results related to influence of CMV infection on T cells subpopulations' counts were represented in figure **5**. We assisted to an increase in total lymphocyte counts especially with couple D+/R+ compared to D-/R- respectively at 3 and 6 months post-BMT reaching statistical significance at day 90(TCD3: $3x10^3$ /µ vs $1x10^3$ /µ cells, $p<0.04$) and at day 180 (TCD3: $4x10^3$ /µ vs $1x10^3$ /µ cells, $p<0.01$),

Moreover, we noticed persistence at higher counts of CD8+ T cells mainly with the couple D+/R+ compared to D-/R- throughout 1 year post-transplantation with higher significant difference (TCD3: $2x10^3$/µ vs $1x10^3$/µ cells, $p<0.05$). This led us consider that CMV infection accelerates CD8+ T lymphocyte reconstitution.

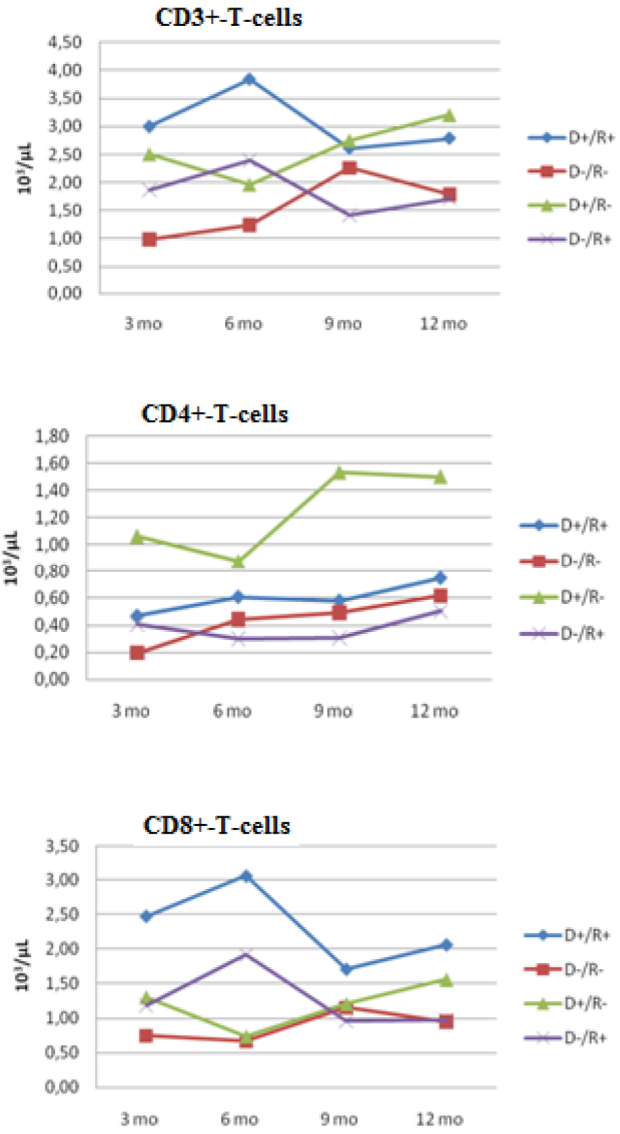

Figure 5: Circulating T cells subpopulations counts (CD3+, CD4+ and CD8+) regarding CMV infection, throughout 1 year post allogenic HSC transplantation.

While we found high significant differences of CD4+ T cells counts with couple D+/R- compared to D-/R- at 3 months (TCD4+: $1x10^3$/µ vs $0.2x10^3$/µ cells, $p<0.01$), 9 months (TCD4+: $1.55x10^3$ /µ vs $0.5x10^3$ /µ cells, $p<0.01$) and at 12 months post-transplantation (TCD4+: $1.45x10^3$ /µ vs $0.6x10^3$/µ cells, $p<0.01$).

3.1.5. Impact of the Amount of CD34+ Cells Injected

The quantity of CD34+ (> $6x10^6$/kg received by patients was significantly associated with the faster recovery of a CD4+/CD8 +(>1ratio) compared to patients who received a lesser amount of CD34 +

cells(<6x10^6/kg): average 5.5 ± 5 months vs 14.8 ± 5 months, p<0.03. Similarly, in children receiving a conditioning regimen including anti-lymphocyte serum (ALS), the CD4+/CD8+>1 ratio was reached more rapidly compared in patients who have received a conditioning without anti- lymphocyte serum (average of 5.8 ± 5.5 months vs 14.6 ± 5.2 months, p<0.044).

In our study population, 9 children were evaluable with a follow-up duration greater than 1 year, the recovery of T lymphocytes CD4+/CD8(+>1)ratio was obtained within an average of 10.5 ± 6.8 months.

The bone marrow "Sex matched" reported in 10 patients was significantly associated with the most rapid CD8+ T cells reconstitution compared to recipients with "mismatched" sex (average of 1.29 ± 0.4 months vs 2.56 ± 1.4 months, p < 0.042) with an average of 1.29 ± 0.4 months. While it was about 2.56 ± 1.4 months in recipients with "mismatched" sex.

A quantity of CD34+(> 8x10^6/kg) received by 6 patients was significantly associated with the faster recovery of a CD4+/CD8+(> 1 ratio) compared to patients who received a lesser amount of CD34 + cells (<6x10^6/kg) with an average (5 ± 5 months vs 14 ± 5 months, p < 0.03). Similarly, in children receiving a conditioning regimen including anti lymphocyte serum (ALS), the CD4+/CD8 (+>1) ratio was reached more rapidly (average of 5.8 ± 5.5 months vs 14.6 ± 5.5 months in patients who have received a conditioning without serum anti lymphocyte, p<0.03).

3.1.6. Impact of GVHD Occurrence

The occurrence of acute GVHD was observed in 5 patients among them 2 patients have progressed to chronic GVHD. We observed a negative correlation between the occurrence of GVHD and obtaining a normal CD4+/CD8 + T cell ratio which tend to the significance Indeed, the median time of obtaining a CD4 + / CD8 ratio was about 18 ± 8.4 months compared recipients who presented a GVHD while it was about 8.5 ± 5.2 months in patients who did not present such complication (p < 0.03). Other studied factors including ABO incompatibility, the presence of HLA antibodies etc, did not influence significantly this reconstitution.

4. DISCUSSION

The impaired T cell functions [12] of transplant recipients are thought to be mostly due to some unknown qualitative rather than quantitative defect in the circulating lymphocytes.

Phenotypic analysis of PBMC demonstrated that within 3-6 months various cell subpopulations recover and most return to normal levels within 12 months post allogeneic transplantation [13, 14, 15]. Following conditioning regimen, our FACS analysis of PBMC is in accordance with data published previously, showing a decrease in the absolute number of CD4+ cells accompanied by an increase in CD8+ cells which led to an inverted CD4/CD8 cell ratio [15, 16, 17, 18, 19, 20].

In the present study we reported that within two weeks post- BMT transplantation, all immune system lineages are affected, then we assisted to a quickly reconstitution of neutrophils that control bacterial infections. Thus, the median time for polymorpho nuclear neutrophils recovery to achieve at a normal rate of 500/mm^3, takes on average of almost 3 weeks after BMT [21]. Complete immune reconstitution takes time [22]. First of all it began with T cell regeneration with the expansion of mature T lymphocytes from the graft or from the host more than differentiation or maturation of the rein fused progenitors [23]. The number of circulating lymphocytes CD4+are very low during the first three months and are recovering very slowly then achieve the normal rate 20 years after transplantation. Whereas, those of the CD8+ T cells, reached their normal levels one year after BMT [21]. It is well known that the reconstitution of T cell subpopulations after bone marrow transplantation is unbalanced [23]. The absolute number of T cells CD4+ is below its normal rate during the first six months post transplant, while it is normal for CD8+ T cells. Overall, this lack of T cell reconstitution leads to the susceptibility to opportunistic infections and in many cases to develop autoimmune disorders. We have assisted to this imbalance in our patients. Indeed, the restoration of a normal CD4+/CD8+ T cells ratio was observed after a mean period of 12 months.

During our study many factors are found influencing the immune reorganization. Interestingly, Methotrexate used in the prophylaxis of GVHD was found to affect negatively the aplasia exit. When analyzing the contribution of Methotrexate in the occurrence of mucositis after BMT, we found a significant delay in the release of aplasia in the group of patients receiving Methotrexate. Another factor is largely known to affect immune reconstitution is the amounts of CD34 + cells reinjected or contained in the graft which play a central role within the period of immune reconstitution [25]. In our series, our results corroborate with this hypothesis confirming that a high amount of CD34+ cells was significantly associated with a longer and faster

recovery. Then, another parameter is the rapid lymphocyte CD4+/CD8+>1 ratio.

When interesting to the impact of conditioning chemotherapy, we've found that in the literature most comparisons are essentially made between the conventional conditioning versus non-myeloablative conditioning. So, according to Schulenburg, in a study enrolled 66 patients, the recovery of CD4+T cells is more rapid with non-myeloablative conditioning, while the emergence of B cells is more rapid after conventional chemotherapy [26]. In our study, lymphocyte recovery was significantly faster in patients receiving the antiserum lymphocytes (ALS) used as immunosuppressive. This result may be related to the anti rejection due to ALS reinjection.

Other factors appear to influence the immune recovery including, the age of the recipient because thymic function decreases with age starting from 25 years [26,27,28,29]. Interestingly, the recovery rate of CD4 + T naive cells is much slower in adults than in children, whereas recipient age does not seem to influence the delay of reappearance of T lymphocytes CD8 + [28]. Another factor, the occurrence of CMV infection is considered as an accelerator of CD8+ T cells recovery in our series of patients.

In this work, the link between the age of the recipient and immune reconstitution has been discussed although our cohort is composed exclusively of children, we have found as we've already mentioned that younger age especially of the recipient is associated with less GVHD and higher recovery of CD4+ T cells. In general, children have a lower incidence of severe GVHD compared with adults. The manipulation of the graft accounts among the factors influencing the immune reconstitution. So, following depletion of CD8+ T cells was found to reduce CD8+ T cell reconstitution during the six months following transplantation of hematopoietic stem cells [23]. Another factor was also observed to affect such reorganization is the occurrence of GVHD. In fact, thymic function seems to be deteriorated by the GVH occurrence [27,29].

A study conducted by Autran *et al*, have reported that adults patients normalize their CD4+/CD8+ ratio one year after bone marrow transplantation, except in patients with chronic GVHD [22]. In another study conducted by Bertrand Y and Fischer A, they have demonstrated that the occurrence of acute GVHD did not influence the development of immune T functions over a year after transplantation, whereas chronic GVHD had a negative influence on reconstituting T cells [30]. This parameter was studied in our study and wasn't found to be significant probably because of small sample sizes.

Hence, a better understanding of engraftment, immune reconstitution, tolerance, and graft-versus-host disease (GVHD) as well as improved transplant strategies and supportive care have been major reasons for the success of HSCT as potentially curative therapy for many disorders in children.

CONCLUSION

Restoration of immune function constitutes a significant problem for patients following an HCT. In our study, we found that in pediatric patients, the immune reconstitution post-BMT began with the myeloid lineage and lymphoid B and T lineages. Inversion of the CD4+/CD8+ ratio is invariably observed following HCT in the majority of patients and it's not due to a thymic defect. Particularly, the CD4 + / CD8 + ratio is more faster if the standardized graft is richer in CD34+ cells and even more, purified selected HSC graft demonstrate reversed CD4+/CD8+ ratios. Moreover, to improve T cell survives the presence of cells expressing MHC class II and I was required. But, unfortunately most cells expressing high levels of MHC class I are acutely radiosensitive and chemotherapy sensitive.

Nevertheless, delayed immune reconstitution and disease relapse remain major limitations to the widespread application of allogeneic transplantation. Understanding the causes of immune escape and hampered immune reconstitution provides the rationale to develop powerful immunotherapies.

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