Prospective Study of the Regeneration of B cells after Allogeneic Hematopoietic Stem cell Transplantation

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الملخص:

تجدد الخلايا البائية بعد زراعة الخلايا الجذعية الدموية متغير ويتاثر بعدة عوامل مثل عمر المريض، المرض الأساس، مرض رفض خلايا جسم العائل للخلايا المزروعة وعلاجه، أجريت هذه الدراسة في الفترة بين اكتوبر2009 الى اكتوبر 2010. اجريت دراسة استباقية لتحليل بعض أنواع الخلايا البائية وتجددها عند أربعة عشر مريضا متتاليا خضعوا لعمليات زراعة الخلايا الجذعية، تم تحليل الخلايا البائية قبل الزراعة وأثناء السنة الأولى من الزراعة وذلك عبر قياس الخلايا البائية الحاملة لمستقبل سي دي 19+ وسي دي20+ لكل مريض، البيانات تم التعبير عنها بشكل القيمة الأدنى ، القيمة الأعلى و المتوسط الحسابي باستخدام برنامج الاكسيل، وجد أن هناك زيادة متدرجة في العدد المطلق للخلايا البائية الحاملة للمستقبل سي دي 19+ وسي دي20+. معدل الخلايا البائية كان مرتفعا بشكل مميز عند المرضى الذين لم يتكون لديهم مرض رفض خلايا جسم العائل للخلايا المزروعة المزمن، تجدد تكون الخلايا البائية بعد زراعة الخلايا الجذعية الدموية متغير ويتاثر بعوامل متعددة من أبرزها رفض خلايا جسم العائل للخلايا المزروعة المزمن.

Abstract:

B cells reconstitution after hematopoietic stem cell transplantation (HSCT) is variable and influenced by different parameters such as patient age, underlying disease, and graft versus host disease (GVHD) and its therapy. This study was carried in the period from October 2009 to October 2010.we carried out a prospective analysis of different B-cell subsets regeneration in 14 consecutive HSCT recipients before and during the first year post transplantation by measuring CD19+ and CD20+ B-cell subsets for each patient. Data were

presented as minimum, maximum and median using Excel spread data sheath. Gradual increasing of the absolute count CD19+ and CD20+ B-cells subsets was demonstrated. Frequencies of B-cells were significantly higher in patients without chronic GVHD. B-cells reconstitution after HSCT is changeable and affected by many factors especially chronic graft versus host disease.

Keywords: Allogeneic Hematopoietic stem cell transplantation, B cells, Reconstitution, graft versus host disease

1-Introduction:

Allogeneic hematopoietic stem cells transplantation (HSCT) considered as a strong curative therapy for a different types of immunodeficiency, metabolic disorders, and haematopoietic malignancies. By this process, thousands of patients have been heal from their underlying disease. The ability of hematopoietic stem cells transplantation (HSCT) to cure is influenced by many factors (Devine SM, 2003; 141: 7–32) include: the type of disorder(Burnett AK, 1997; 349: 270–275) , patient age, state of disease at the time of transplant (Horowitz MM, 1992; 367–377), stem cell source(Korbling M, 2001; 98: 2900–2908,), human leucocytes antigens (HLA)-matching compatibility between the donor and recipient (Davies SM 1997; 15: 557–565,), (Gustafsson A, 2000; 25: 1059–1065) , pre-transplant conditioning (Michallet M , 2001; 19: 3340–3349),(Morecki S, 2001; 28: 243–249), cell depletion (Ash RC, 1991; 7: 443–452), (Gilmore MJ, 1986; 64: 69–75).,graft versus host disease (GVHD) and its treatment (Mc Glave P, 1993; 81: 543–550), post transplant infections (Hongeng S, 1997; 350: 767–771), (Ochs L, 1995; 86: 3979–3986).

All of these parameters will determine the final outcome of the transplant. After transplantation, there is a period of long immune deficiency which associated with increasing the susceptibility to infection. The Long immune deficiency occurs mainly from deficiencies in effective CD4+ T cell and B cell reconstitution. (Small TN, 1997; 3: 65–75), (Small TN, 1999; 93: 467–480), (Storek J, 2000; 96: 3290–3293).

Deficiency of B cells in peripheral blood detected in the first months after HSCT and affected by chronic graft versus host disease (cGVHD) and / or its therapy (Asma GE, 1987; 43: 865-870), (Leitenberg D, 1994; 102:231-236), (Storek J, 2001; 98: 489-491).

Graft versus host disease occurs when donor T cells react with particular proteins on host cells. The most important proteins are Human Leukocytes Antigens (HLA). These antigens are highly polymorphic and are encoded by the

major histocompatibility complex (MHC). The incidence of acute GVHD is related with the degree of mismatch between HLA proteins (Loiseau P, (2007; 13:965-974), (Ratanatharathorn V, 1998; 92: 2303-2314,). Chronic GVHD is one of the major causes of death following HSCT (Lee S J, 2002; 100:406-414) .It manifest as progressive (active acute GVHD changed into chronic), quiescent (acute GVHD completely resolved but followed later by chronic GVHD) or it may occur de novo.

GVHD leads to immune deficiency after HSCT either by direct attack on the thymic stroma by allogeneic effector cells or through the immunosuppressive effects of the medications that used to prevent or treat GVHD (Sullivan KM, 1992; 10: 127-134).

Different types of procedures are used to assess post-transplant immune recovery, including tests that are done routinely in clinical laboratories (absolute lymphocytes counts, CD4+, CD8+, NK cells, B cells, and antibodies titers (Porrata LF, 2004; 4: 78-85).

Peripheral blood B cell development can be characterized by many surface markers CD19, IgD, CD38, CD20, and CD27 immunophenotyping (Sanz I, 2008; 20:67-82).

CD19 is expressed on the surface of B cells from the first stages of B cells development: Progenitor B cells, pre- B cell, and still expressed on B cells after they have undergone heavy and light chain recombination and express IgM B cell receptor (BCR) on their cell surface, as stage called immature B cells. CD19 continues to be expressed until the cell becomes an immunoglobulin secreting plasma cell. CD20 is first expressed on immature B cells and continues to be expressed until the B cell becomes an immunoglobulin secreting plasma cell (Withers DR, 2007; 109:4856-4864).

2-Methods:

2.1. Patients and setting:

Fourteen consecutive patients are included in this study. All underwent HSCT from October 2009 to October 2010 at the Campus Virchow Clinic, Charite' University, Berlin, Germany. Median Age of donors 30 years, range 7-50 years. Median Age of patients 9 years, range 1-25 years. 57% of patients were male, 43% were female. Thirteen patients (93%) received their grafts from matched unrelated donors (MUD), and one patient (7%) from matched related donors (MRD). All the patients received Aciclovir as Antiviral prophylaxis. All the

patients received Anti Thymocytesglobulin, Cyclosporine-A, and Methotrexate as GVHD prophylaxis.

Underlying disease was in 44% of patients Acute lymphoblastic Leukaemia (ALL), 14% Acute myeloid Leukaemia(AML),14% metabolic disorders, 7% Chronic myeloid Leukaemia (CML),7% Myelodysplastic Syndrome (MDS),7% Fanconi Anemia, and 7% Primary Immune defect.

Type of transplantation was 71% bone marrow and 29% Peripheral Blood stem cell (PBSC).

Twelve of the patients (86%) had acute graft versus host disease, range from grade I – III, Four patients (29%) had grade II of chronic graft versus host disease.

2.2 Sample techniques:

Blood samples were collected once pre-transplant and three, six, nine, and 12 months post transplantation. Informed consent was obtained from all patients or their parents. The Study was done on whole blood.

2.3 Cell Preparation:

Peripheral venous blood from patients monthly after Transplantation was drawn into 10-ml Li-heparin/EDTA vacationer Becton Dickinson (BD, USA) after informed consent.

2.4 Antibodies used:

The following monoclonal antibodies were obtained from Becton Dickinson (BD, USA): Phycoerythrin (PE)-conjugated anti-CD19 (clone J25C1) and Phycoerythrin (PE) -conjugated anti-CD20 (clone L27).

2.5 Assay Principle:

Aliquots of 50 µl 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 fixed with FACS-lysing solution, (BD Pharmingen, USA).

2.6 Flow Cytometry analysis:

Blood samples were stained with fluorochrome-conjugated antibodies against CD19 and CD20 as previously described. Cells were analyzed on FACS CAN (BD, USA) flow cytometer .Data was further analyzed using Cell Quest program software. B cells subset populations (CD19+ and CD20+) for each patient were gated and quantified pre-transplant and three, six, nine, and 12 months after transplant and absolute counts / µl of whole blood were determined.

3. Data analysis:

Descriptive statistics were used to present the results in form of median, minimum and maximum, using Microsoft excel data sheath.

4. Results:

The Reconstitution of B cells expressing CD19+ and CD20+ surface markers was measured in peripheral blood before transplantation (before Radio-chemotherapy) and post transplant blood samples were collected from patients at 3, 6, 9 and 12 months post allogeneic hematopoietic stem cells (HSCT) . Absolute count of CD19+ B cells was measured and presented as median, minimum, and maximum.

Table 1. $CD19^+$ B cells per µl in peripheral Blood of patients before and after allogeneic HSCT (Median, Minimal-/ Maximal values)

Time	Median	Minimum	Maximum
Before Transplantation	450	20	5450
3 months after HSCT	120,	0	587
6 months after HSCT	217	0	854
9 months after HSCT	240	7	860
One Year after HSCT	271	11	1000

As shown in Figure 1, the reconstitution of CD19+ gated B cells was measured before HSCT and every three months post transplantation. There was a gradual increasing in the CD19+ B cells count/µl.

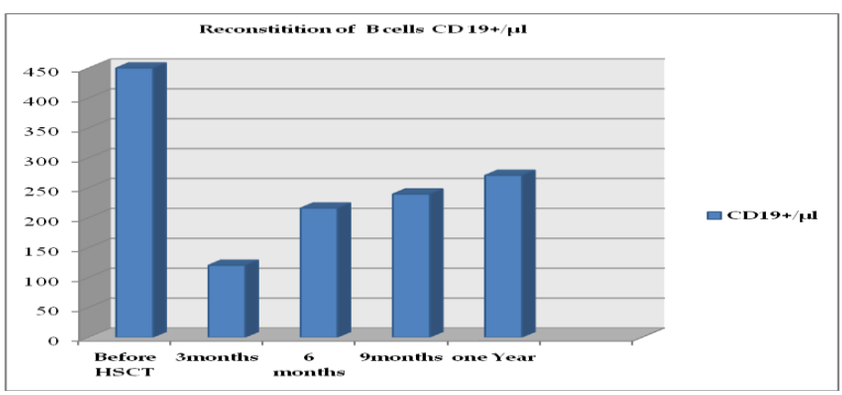

Figure 1. Reconstitution of $CD19^+$ gated B cells per µl

Absolute count of CD20+ B cells was measured and presented as median, minimum, and maximum.

Table 2 $CD20^+$ B cells per µl in peripheral Blood of patients before and after allogeneic HSCT (Median, Minimal-/ Maximal values)

Time	Median	Minimum	Maximum
Before Transplantation	550	15	4320
3 months after HSCT	99	0	538
6 months after HSCT	213	0	823
9 months after HSCT	228	9	830
One Year after HSCT	244	10	850

As shown in Figure 2, the reconstitution of CD20+ gated B cells was measured before HSCT and every three months post transplantation. There was a gradual increasing in the CD20+ B cells count/µl.

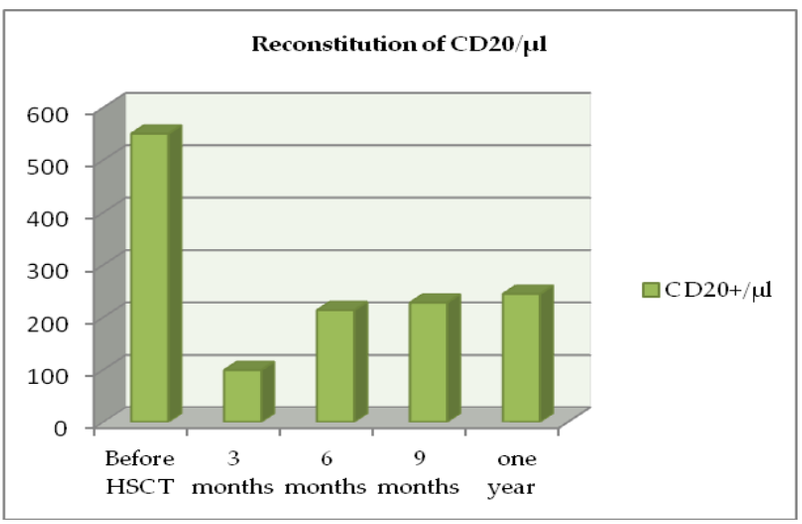

Figure 2. Reconstitution of $CD20^+$ gated B cells per µl

Upon the correlation with the factors that have impact on the regeneration of B cells after allogeneic haematopoitic stem cell transplantation, there was an effect

of graft versus host disease on the absolute count of CD19+ and CD 20 + B cells.

As shown in figure 3, the absolute count of CD19+ and CD 20 + B cells was significantly higher in patients without chronic graft versus host disease compared with patients with chronic graft versus host disease

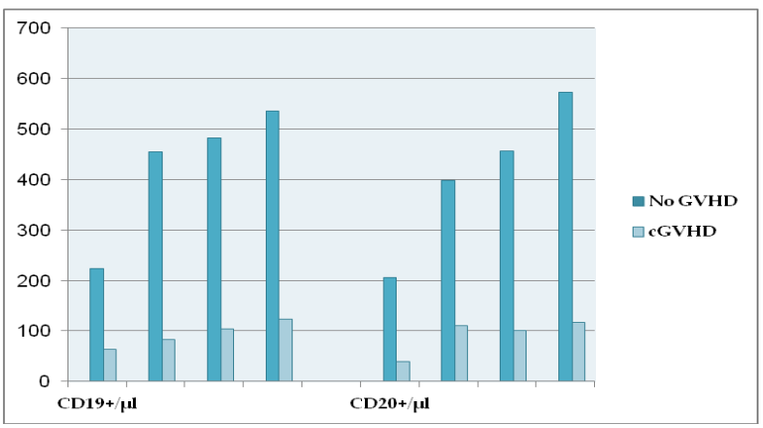

Figure 3. $CD19^+$ B cells per µl and $CD\ 20^+$ B Cells per µl in patients with and without chronic graft versus host disease

6. Discussion:

Allogeneic hematopoietic stem cell transplantation (HSCT) provides a unique chance to improve the development of immune reconstitution. Effective immune recovery and protection from opportunistic infections requires antimicrobial B cells and antibody development. Although the T-cell component of the immune defect has been relatively well defined, the long-term B-cell component is less well known (Small TN, 1999; 2: 467-480), (Storek J. 2001; 97: 3380-3389).

After HSCT, the three main goals that are extremely important for achieving long-term survival in these patients include engraftment of the transfused stem cells, prevention of graft versus host disease (GVHD) and neogenesis of functionally diverse and matured T and B cells (Cuvelier GD, 2009;13: 179-188).

The kinetics of early T and B cell recovery after HSCT, occurring during the first three months post allogeneic hematopoietic stem cells (HSCT), has a major impact on achieving these goals.

The thymus and the bone marrow are the primary anatomic sites for T and B cell neogenesis from undifferentiated hematopoietic progenitor cells. Within these organs, hematopoietic progenitor cells that have been committed to the T

and B cell lineage undergo rapid proliferation and differentiation to mature cells. During this process, a diverse receptor repertoire is formed, and the resulting cells are able to respond to a wide array of internally and externally processed antigens (Nossal GJ, 1994; 76:229-239), (Fry AM, 1989; 246:1044-1046), (Hodes RJ, 1989; 246:1041-1044).

Ideally, T and B cells should regenerate from stem cells present in the graft, and various HSCT procedure manipulations, such as pre- transplant conditioning therapy, are performed in order to ensure its occurrence. Donor hematopoietic stem cells differentiate and proliferate to repopulate all blood lineages providing normal cell numbers of red blood cells, platelets, and neutrophils. Successful immune reconstitution and protection from infection requires antimicrobial B cell and antibody development. Studies of B cell reconstitution after hematopoietic stem cell transplantation have primarily examined immunoglobulin concentration, B cell quantification, and antimicrobial antibody development in relation to donor/recipient serologic status or vaccination. Following HSCT, humoral immunity has three distinct contributions. First, recipient antibody persists with an average half-life of 30-60 days, and some recipient plasma cells persist for years following allogeneic HSCT (Van Tol MJ et al) providing protective antimicrobial humoral immunity (Wimperis J Z, 1987; 138: 2445-2450).

Some recipient anti-donor alloimmune responses are detrimental contributing to primary graft rejection (Petersdorf EW, 2001; 345:1794-1800), (Taylor PA, 2007; 109:1307-1315) and prolonged red cell aplasia when donors and recipients are ABO major mismatched (Bolan CD , 2001; 98:1687-1694), (Griffith LM, 2005; 128:668-675.) Second, donor grafts contain naive and memory B cells that have already undergone positive and negative selection in the HLA-identical donor and contribute adoptive antimicrobial and alloreactive B cells. Third, B cells reconstituting from donor hematopoietic stem cell recognizing disparate recipient antigens as" self", will be clonally deleted preventing allorecative responses, but remain capable of responding to infectious challenges and vaccinations.

Delayed immune reconstitution after HSCT has been associated with significant morbidity and mortality, especially after allogeneic HSCT (allo-HSCT), including infections and relapse (Pizzo PA , 1994; 84:2221-2228),(Mackall CL, 1994; 84:2221-2228),(Mackall CL,2000; 18:10-18).

In particular T-cell immunity is affected by the combined effects of the conditioning regimen, thymic involution in the host, donor age, type of graft, stem cell dose, ex vivo or in vivo T-cell depletion, donor-host disparity, graft-versus-host disease (GVHD) prophylaxis, and GVHD itself (both acute and chronic).

Innate immunity recovers in the first months after HSCT: first monocytes, followed by granulocytes and natural killer cells (Storek J, 2008; 30:425-437). In contrast, adaptive immunity, which consists of cellular (T lymphocytes) and humoral (B lymphocytes) immunity, takes 1-2 years to recover and a significant number of patients will incur even longer-lasting deficits (Mackall Cl, 1995; 332: 143-149), (Hakim FT, 2005; 115: 930- 939).

B cell counts recover by 6 months after auto HSCT and by 9 months after allo-HSCT. Recovery of humoral immunity is: (1) initially impaired because of limited antibody repertoire, (2) dependent on T cell help, and (3) decreased due to GVHD prophylaxis and treatment, and GVHD itself (Storek J, 2001; 98:489-491).

The use of multiparameter flow cytometry enables identification of additional subsets of T, B, and NK cells, as well as myeloid subsets such as dendritic cells (DC). There was an impact of chronic graft-versus-host disease (GVHD) on B cell subset recovery. CD19+ and CD20+ B cells were decreased in patients with symptoms of chronic GVHD. Quantitative B cell deficiency in peripheral blood has long been noted in the first months after HSCT and can persist for years being worsened by chronic GVHD and /or the treatment of chronic GVHD. In chronic GVHD (cGVHD), poor B cell reconstitution seems mainly due to reduced numbers of B cell progenitors and un-switched memory B cells (Abrahamsen I W, 2005;90:86-93.), (Hilgendorf I, 2012; 25:87-96,).

7. Conclusion:

B cell reconstitution after allogeneic hematopoietic stem cell transplantation (HSCT) is variable and influenced by different patient, donor, and treatment related factors. In this work, we had described B cell reconstitution after allogeneic hematopoietic stem cell transplantation, including the kinetics of reconstitution of the different B cell subsets and the development of the B cell repertoire, and discussed the impact of chronic graft versus host disease on the B cell recovery.

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