INTRODUCTION

During the last 3 decades, bone marrow transplantation (BMT) and hematopoietic stem cell transplantation (HSCT) have become the treatment of choice for patients suffering from certain malignant and non-malignant hematological disorders ***(Jones, 2010),*** such as , severe aplastic anemia, severe combined immunodeficiency, or hemoglobino-pathies ***(Felfly and Haddad, 2014).***

For patients who had undergone allogenic HSCT monitoring for minimal residual disease and recurrent malignancy using chimerism testing should be performed at least monthly duringthe first year ***(McClune et al., 2012).***

Different types of chimerism are known including complete and mixed chimerism. Depending on the immunogenetic differences between the donor and recipient, patients who show no evidence of host DNA at any time during the post-transplant follow-up are considered to be in "Complete Chimerism" (CC). CC is usually accepted to be associated with Severe GVHD, low risk of relapse and better prognosis ***(Yeliz et al., 2007)***. Patients with donor and recipient DNA in any of the samples were defined as having mixed chimerism (MC). MC indicates the presence of both donor and recipient cells within a given cellular compartment. It associates with less graft versus host disease (GVHD) and graft versus leukemia

(GVL) effect, higher frequency of relapse and shorter disease-free survival compared to CC ***(Pidala et al., 2014).***

Chimerism is done using PCR amplification of many highly polymorphicvariable number tandem repeats (VNTR) loci as: ApoB, D1S80, YNZ-22 and 33.6 ***(Daud et al., 2010)***. Other hypervariable minisatellite loci also exist as (MS31A (D7S21), MS205 (D16S309), CEBl (D2S90), g3 (D7S22), YNH24 (D2S44), B6.7 and the insulin minisatellite ***(Kletzel et al., 2013).***

Whole blood chimerism analysis has a high discrimination rate but a limited sensitivity of 1-5%. If blood components are sorted to their specific lineages the sensitivity increases 10-100 folds. Thus, lineage specific chimerism can be used to reveal mixed chimerism in specific leukocyte population that is masked in the whole blood analysis. It can provide information about reoccurrence of the original clonal disease ***(Jens et al., 2012).***

Selection of appropriate cell populations (T-cells, myeloid cells, etc) is first performed and the individual populations are subsequently subjected to chimerism analysis ***(Haspel et al., 2008).*** Different cell subpopulations engraft at different rates, with myeloid engraftment usually occurring prior to T-cell engraftment ***(Shay and Kang, 2013).***

Assessment of lineage specific chimerism after BMT is carried out in both myeloid and lymphoid malignancies such as CML, AML, CLL and ALL. Information about the relative proportions of donor and recipient T Cells is important in understanding the dynamics of engraftment and predicting graft versus leukemia and graft versus host effects ***(Jens et al., 2012).*** Monitoring of natural killer (NK) cell chimerism may also provide unique prognostic information ***(Chen et al., 2008).*** High donor T-cell and NK -cell chimerism appears to be critical for successful engraftment, which in turn is strongly associated with sustained antitumor response ***(Cheng et al., 2013).***

AIM OF THE WORK

The main goal of this study is to analyze the evolution of chimerism by using lineage specific chimerism analysis and to compare it with whole blood chimerism analysis method.

Also, the usage of a semi-quantification method in case of mixed chimerism to predict the outcome and enabling rapid interference in case of relapse or graft rejection is evaluated.

OVERVIEW ON CHIMERISM

Hematopoietic stem cell transplantation (HSCT) has advanced into an effective strategy for the management of hematological and oncological disorders ***(Burt et al., 2008)***. It involves the transfer of multipotent hematopoietic stem cells, usually attained from bone marrow, peripheral blood, or umbilical cord blood aiming at restoring normal heamatopoiesis. Radiotherapy and chemotherapy are recommended as conditioning regimens prior to BMT to inhibit the host immunity and reduce tumor burden ***(Park et al., 2015).***

Graft types:

Graft types are either autologous or allogeneic transplants. Autologous HSCT involves apheresis of haematopoietic stem cells (HSC) from the patient and storage of the harvested cells. The patient is then subjected to high-dose chemotherapy with or without radiotherapy with the purpose of eliminating the patient's malignant cell population at the cost of partial or complete bone marrow aplasia. The patient's own stored stem cells are then transferred into his/her bloodstream, where they substitute destroyed tissue and resume the patient's normal blood cell population ***(Bruno et al., 2007)***. Autologous transplants have the advantage of lower possibility of infection during the immune-compromised period of the treatment since the recovery of immune function is rapid. Also, the percentage of patients experiencing rejection (graft-versus-host disease) is

extremely low due to the donor and recipient being the same individual ***(Felfly and Haddad, 2014).***

These advantages have proven autologous HSCT as one of the standard second-line treatments for some diseases as lymphoma ***(Bruno et al., 2007)***. However, for others such as acute myeloid leukemia, the lowered mortality of the autogenous relative to allogeneic HSCT may be compensated by an increased likelihood of cancer relapse and related mortality, and therefore the allogeneic treatment may be favored for those conditions ***(Schold et al., 2011).***

However, allogeneic HSCT consists of two people: the donor and the recipient. Allogeneic HSC donors need to have a tissue (HLA) type that matches the recipient. Matching is done on the basis of variability at three or more loci of the HLA genetic sequence, and a perfect match at these loci is preferred ***(Nivison-Smith et al., 2005)***. Even if there is a good match at these significant alleles, the recipient will require immuno-suppressive medications to diminish graft-versus-host disease.

Allogeneic transplant donors may be related (usually a strictly HLA matched sibling), syngeneic (a monozygotic or 'identical' twin of the patient - necessarily particularly rare since few patients have an identical twin, but proposing a source of perfectly HLA matched stem cells) or unrelated (donor who is not related and found to have very close grade of HLA matching). Allogeneic transplants are also achieved by using

umbilical cord blood as the source of stem cells. Broadly, by transfusing healthy stem cells to the recipient's blood stream to reconstitute a healthy immune system, allogeneic HSCTs appear to improve probabilities for cure or long-term remission once the direct transplant-related complications are resolved ***(Park et al., 2015).***

A well-matched donor is found by doing HLA-testing from the blood of potential donors. The HLA genes fall in two classes (Type I and Type II). In general, mismatches of the type-I genes (i.e. HLA-A, HLA-B, or HLA-C) raises the risk of graft rejection. A mismatch of an HLA Type II gene (i.e. HLA-DR, or HLA-DQB1) raises the risk of graft-versus-host disease. In addition a genetic mismatch as minor as a single DNAbase pair is significant so perfect matches necessitates knowledge of the particular DNA sequence of these genes for both donor and recipient. Leading transplant centers presently perform testing for all six of these HLA genes before asserting that a donor and recipient are HLA-identical ***(Simaria et al., 2013)***.

Indications for HSCT:

HSCT was initially developed to treat congenital immunodeficiencies and non-malignant hematological conditions such as (β thalassemia major (Cooley's anemia), aplastic anemia, Fanconi anemia, sickle-cell disease and hemoglopinopathies) ***(Hütter et al., 2009).*** It has also become a potent strategy for treating autoimmune and metabolic diseases

(Mahmoud et al., 2015). Diseases commonly encountered in HSCT are listed in Table 1.

Table (1): Diseases commonly encountered in HSCT.

Autologous BMT	Allogeneic BMT
Multiple myeloma (MM). Non-Hodgkin lymphoma (NHL). Hodgkin disease (HD). Acute myeloid leukemia (AML). Neuroblastoma. Germ cell tumors. Autoimmune disorders.	Acute myeloid leukemia (AML). Acute lymphoblastic leukemia (ALL). Chronic myeloid leukemia (CML). Chronic lymphocytic leukemia (CLL). Myeloproliferative disorders. Myelodysplastic syndromes (MDS). Multiple myeloma (MM). Non-Hodgkin lymphoma (NHL). Hodgkin disease (HD). Aplastic anemia (AA).

Note: Multiple myeloma remains to be the most common indication for autotransplantation and acute myeloid leukemia for allogeneic transplantation ***(Passweg et al., 2012).***

Conditioning regimens:

Because the majority of HSCT techniques are performed for the treatment of malignant diseases, the conditioning regimens might be used to provide tumor cytoreduction and perfectly disease eradication ***(Welniak et al., 2007).*** The therapeutic effects of HSCT on malignancies are also mediated via generation of the graft versus tumor effect by immunocompetent cells in the graft. Conditioning regimens that can reduce graft versus host disease without affecting

engraftment and graft versus tumor effects are being discovered ***(Shi et al., 2013).***

The intensity of the conditioning regimens differs significantly ***(Gyurkocza and Sandmaier, 2014).*** Based on the expected duration and reversibility of cytopenia during post-transplant period, at (2009), Bacigalupo et alcategorized the conditioning regimens into two categories, i.e., myeloablative and reduced-intensity or non myeloablative conditioning regimens.

Myeloablative versus reduced-intensity or non myeloablative conditioning regimens:

Myeloablative conditioning regimens end in irreversible cytopenia, and stem cell support is required after HSCT. Whereas both non myeloablative conditioning or reduced-intensity conditioning regimens share one important advantage, i.e., both end in reversible myelosuppression (usually within 28 days) when given without stem cell support. Moreover, these methods use lower doses of cytoreductive treatments and result in low non hematological mortality ***(Shi et al., 2013).***

Reduced-intensity and non myeloablative conditioning regimens may decrease the risk of severe acute graft versus host disease ***(Ferrara et al., 2009)***. These regimens cause only partial host damage, which may consequently translate into less release of inflammatory cytokines which, it has been suggested,

provide a pro inflammatory milieu for establishment of graft versus host disease ***(Beres and Drobysk, 2013).***

In addition, development of transient mixed donor-host chimerism after reduced-intensity and non myeloablative conditioning regimens may facilitate the establishment of mutual tolerance, which in turn down regulates the activity of graft versus host disease ***(Beres et al., 2012).***

Therefore, donor lymphocyte infusion (DLI), which has been used as a helpful tool for inducing a sustained complete response of malignancies, could replace high-dose cytotoxic therapy because of its graft versus tumor effects in reduced-intensity and non myeloablative conditioning regimens instead of being always followed by serious graft versus host disease in myeloablative conditioning regimens ***(Pingali and Champlin, 2015).***

Donor lymphocyte infusion performed after these conditioning regimens has shown promising results, even in the treatment of solid malignancies ***(Kami et al., 2004)***. DLI may be amplified by activation of donor lymphocytes with IL-2 or in vivo administration of IL-2. Identification of tumor antigens will lead the way to ex-vivo generation and expansion of tumor specific cytotoxic T-lymphocytes to be used as potent immunotherapy without the hazards of GVHD ***(Gajewski et al., 2013).***

Also, granulocyte colony-stimulating factor has been used with standard dose of cyclosporin A as the sole anti-graft-versus-host disease (GVHD) prophylaxis which resulted in stable partial or complete chimerism ***(Slavin et al., 1998)***. Transient mixed chimerism which may protect the host from severe acute GVHD may be successfully reversed post allogeneic HSCT with graded increments of donor lymphocyte infusions, thus resulting in eradication of malignant or genetically abnormal progenitor cells of host origin ***(Reilly et al., 2015).***

Adoptive immunotherapy with DLI (DCI):

A DCI is a form of cellular therapy that uses cells from the original donor, and is commonly used to create a graft-versus-leukemia/ tumor (GVL/GVT) effect ***(Slavin et al., 2001)***. The types of cells used in a DCI include, but are not limited to: lymphocytes, unstimulated peripheral blood mononuclear cells, dendritic cells, and/or mesenchymal cells ***(LeMaistre et al., 2013).***

The curative effect of allogeneic HSCT is based largely on the alloreactivity of donor lymphocytes, which mediate a graft-versus-leukemia (GVL) effect ***(Dazzi and Goldman, 1999)***. This GVL effect is associated with the presence of graft-versus-host disease (GVHD) and is influenced by the degree of major histocompatibility complex (MHC) disparity and the presence of T lymphocytes within the graft, suggesting that it is

mediated largely by graft-versus-host (GVH) alloreactive donor T cells. Unfortunately, this beneficial GVL effect is often counterbalanced by the mortality and morbidity associated with GVHD ***(Warren and Deeg, 2013).***

GVL activity was markedly superior in mixed chimeras compared to full chimeras, demonstrating the importance of host-type APCs for the induction of maximal GVL effects ***(Bethge et al., 2004)***. This effect was dependent on MHC class I expression on host APCs ***(Leveque et al., 2015).***

The maximal GVL effects observed in mixed chimeras can occur without GVHD, despite mediation by a potent GVH alloresponse. The avoidance of GVHD following DLI requires 2 preconditions. The first precondition is sufficient time for the recipient to recover from conditioning-induced inflammation that may promote the migration of T cells into GVHD target tissues. Because this interval has been shown to allow DLI to convert mixed chimerism to full chimerism without GVHD. The second precondition is an absence of GVH-reactive T cells in the initial donor transplant ***(Loren and Porter, 2006)***. The presence of such early GVH alloreactions, in combination with early conditioning-induced inflammation, might produce clinical or subclinical GVHD, with target tissue inflammation, that could lead to severe GVHD following DLI. This GVL effect is mediated by CD8+ T cells without any apparent role for CD4+cells ***(Yang et al., 2015).***

DLI is effective in generating anti-tumor responses, especially for relapsed chronic-phase CML. Response rates and durability appear lower with myeloma, AML and myelodysplastic syndrome, and minimal with ALL ***(Peggs and Mackinnon, 2001).*** The capacity of CML cells to function as professional APCs and the failure of other tumor types to do so may account for these observations ***(Mawad et al., 2013).*** Other studies suggest that the provision of a host-type professional APC population in the form of mixed chimerism could augment the ability to achieve GVL effects from DLI in patients with tumors that have poor APC capacity. Results in patients with refractory lymphomas following non myeloablative HSCT that induces mixed chimerism are consistent with this possibility ***(Kamimura et al., 2012).***

Early experience established that treatment using DLI following hematologic relapse resulted in a significant incidence of marrow a plasia, resultant on an immune-mediated destruction of the reconstituted host hematopoeisis ***(Porter et al., 1999).*** Treatment earlier in the course of relapse (at the cytogenetic or molecular level) largely avoided this complication because donor hematopoiesis remained intact ***(Takami et al., 2014).*** Further evidence that the immune-mediated effect may not be truly tumor specific but is rather aimed at antigenic determinants of the host lymphohematopoietic system came from studies of lineage-specific chimerism. Mixed chimerism in T cells, a compartment that would not be

predicted to be involved in the neoplastic process in chronic myeloid leukemia (CML), was shown to be an independent marker for subsequent disease relapse/progression. Administration of DLI could target this compartment leading to conversion to full donor chimerism ***(Reshef et al., 2014).***

Graft-versus-host Disease (GVHD) and Graft versus Leukemia/Tumor (GVL) effect:

GVHD is an immunological phenomenon resulting from the reaction of donor immune cells against major or minor histocompatibility antigens of the recipient ***(Goker et al., 2001)***. GVHD is primarily caused by donor-derived T-cells ***(Beres and Drobysk, 2013).***

In the past, GVHD was classified as acute or chronic based on its time to diagnosis following transplant, and other clinical and histological (biopsy or post-mortem) features ***(Lee et al., 2003)***. Today, there has been increased recognition that acute and chronic GVHD are not dependent upon the time since HSCT. However, organ staging and overall grade should only be calculated from the clinical picture, not histology ***(Jagasia et al., 2015).***

A GVL effect can be obtained by transfusion-induced suppression of the host's hematopoiesis resulting from sharing of histocompatibility antigens with the leukemia ***(Bleakley and Riddell, 2004)***. The primary targets of graft-versus-host

reactions are cells of the hematopoietic system of the host. Several minor histocompatibility antigens (mHA) with a tissue distribution restricted to hematopoietic cells have been described that may elicit strong GVL effects. This is done through stimulation of Donor T cells by dendritic cells (DCs) of the host, through presentation of mHA. The best characterized minor antigens are the Y chromosome derived HY peptide and the autosomal HA1 to HA5 peptides. Minor histocompatibility antigens such as HA1 and HA2 have restricted tissue distribution and are present normally only on haematopoietic cells. Others such as HY are more universally distributed, expressed for instance on gut epithelium. HA1 and HA2 are expressed on leukemic cells and some tumor cells, making them potential targets for cellular therapy ***(Holtan et al., 2014).***

Dendritic cells (DCs) of leukemia origin have been demonstrated in CML, and they may present mHA and leukemia-specific peptides to donor lymphocytes ***(Clark et al., 2001)***. Most probably, the spontaneous differentiation of CML progenitor cells to DCs is one important factor in the good response of CML to DLT ***(Garber et al., 2014).***

Similarly, blasts of acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) may differentiate spontaneously to Dcs, or differentiation can usually be induced by stimulation with granulocyte macrophage-colony stimulating factor (GM-CSF) alone or in combination with other cytokines ***(Schmetzer et al., 2007)***. In the presence of cytokines,