UNIVERSITI SAINS MALAYSIA

QUALITY OF STORED CORD BLOOD IN NATIONAL BLOOD CENTRE BASED ON MATERNAL AND NEONATAL FACTORS

By,

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DECLARATION

I hereby declare that this thesis represents my own work and all the sources had been quoted and acknowledged by means of complete references. This thesis has been sent to Universiti Sains Malaysia for the degree of Masters of Medicine in Transfusion Medicine and it is not to be sent to any other universities. With that, this research might be used for consultation and will be photocopied as reference.

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LIST OF ABBREVIATION

BM	Bone marrow
BMT	Bone marrow transplantation
CB	Cord blood
CBU	Cord blood unit
CFU	Colony forming unit
CIBMTR	Centre for International Blood and Marrow Transplant Research
ESC	Embryonic stem cell
GVHD	Graft versus host disease
GVL	Graft versus leukaemia
HLA	Human leucocyte antigen
HSC	Haematopoietic stem cell
HSCT	Haematopoietic stem cell transplantation
LFS	Leukaemia free survival
MDS	Myelodysplasia
MHC	Major histocompatibility complex
MSC	Mesenchymal stem sell
MUD	Matched unrelated donor
NBC	National Blood Centre
NMDP	National Marrow Donor Program
NSCCC	National Stem Cell Coordinating Centre
TNC	Total nucleated cell
UCB	Umbilical cord blood
UCBT	Umbilical cord blood transplantation

Abstrak

Latar Belakang: Darah tali pusat (UCB) merupakan sumber alternatif kepada sel tunjang alogenik hematopoetik bagi tujuan pemindahan. Kualiti darah tali pusat yang dikumpulkan bergantung pada kandungan hematopoetiknya yang secara langsung mempengaruhi hasil *'engraftment'* dan terapeutik. Demi memastikan kualiti darah tali pusat yang dikumpulkan terjamin, kriteria penerimaan darah tali pusat telah diwujudkan di bank tali pusat, Pusat Darah Negara (PDN) yang bersandarkan piawaian antarabangsa. Namun ia telah menyebabkan kadar penyingkiran unit darah tali pusat yang dikumpulkan agak tinggi. Kajian ini bertujuan untuk mengenalpasti hubungkait antara faktor penderma iaitu ibu dan bayi yang boleh mempengaruhi kualiti kandungan hematopoetik darah tali pusat untuk membantu dalam pemilihan penderma dan seterusnya mengurangkan pembaziran.

Kaedah/Model: Kajian ini merupakan tinjauan semula kajian keratan rentas yang dijalankan dengan meneliti 339 rekod pendermaan darah tali pusat yang berjaya dikrioawetkan dalam tahun 2014 di PDN, Malaysia. Maklumat kandungan hematopoetik yang dianalisa terhadap darah tali pusat yang dikumpulkan ialah jumlah isipadu darah tali pusat, bilangan sel bernukleus (TNC), kepekatan sel CD34+ dan unit pembentuk koloni (CFU). Faktor ibu yang dianalisa ialah umur, kaum, jumlah kandungan, tempoh kehamilan, dan kumpulan darah manakala ciri-ciri bayi yang dianalisa termasuklah jantina, berat lahir bayi dan kumpulan darah. ANOVA sehala, ujian-t tak bersandar, korelasi Pearson dan regresi linear tunggal digunakan untuk

menentukan perkaitan antara faktor-faktor tersebut dengan kandungan hematopoetik darah tali pusat yang dikumpulkan.

Keputusan: Berdasarkan laporan PDN, hanya 38.85% (692) dari jumlah darah tali pusat yang dikumpulkan (1781) pada tahun 2014 memenuhi syarat penerimaan dan disimpan. Bilangan sel bernukleus yang rendah dan jumlah isipadu darah tali pusat rendah merupakan dua penyebab tertinggi pembuangan darah tali pusat. Analisis faktor ibu dan bayi menunjukkan tempoh kehamilan berkait secara negatif dengan jumlah isipadu darah tali pusat ($r= -0.182$, $p=0.001$). Manakala berat lahir bayi berkait secara positif yang lemah dengan jumlah isipadu darah tali pusat ($r=0.174$, $p=0.001$) dan TNC/unit ($r=0.116$, $p=0.034$). Kandungan hematopoetik kesemuanya adalah berkaitan; jumlah isipadu darah tali pusat dengan TNC/unit ($r=0.594$, $p<0.001$), jumlah isipadu darah tali pusat dengan CD34+/unit ($r=0.364$, $p<0.001$) dan jumlah isipadu darah tali pusat dengan CFU ($r=0.177$, $p< 0.015$).

Kesimpulan: Kajian ini mendapati faktor ibu dan bayi berhubungkait dengan kandungan hematopoetik darah tali pusat. Faktor yang dikenalpasti ialah tempoh kehamilan ibu dan berat lahir bayi mempunyai hubungkait yang signifikan dengan jumlah isipadu darah tali pusat dan bilangan sel bernukleus.

Kata Kunci: Darah tali pusat, pendermaaan, bank darah tali pusat, faktor ibu dan bayi

Abstract

Background: Umbilical cord blood (UCB) is an alternative source of allogeneic haematopoietic stem cells for transplantation. The quality of collected UCB depends on its haematopoietic content which directly influences the engraftment and the therapeutic outcome. To ensure the quality of stored UCB, the acceptance criteria were created at the UCB bank in National Blood Centre (NBC) based on established international standard, which resulted in a high discard rate. This study aims to determine the association of maternal and neonatal factors that could influence the quality of haematopoietic contents of the UCB collections thus guide the choice of donors and reduce wastage.

Methods/Design: This is a retrospective cross-sectional study performed by reviewing records of 339 cord blood donations which were successfully cryopreserved in 2014 at the NBC, Malaysia. The data on stored UCB analysed were the UCB volume, total nucleated cell (TNC) count, CD34+ cells concentration, and colony forming units (CFU). The maternal factors analysed were age, race, gravid status, gestational period and blood group while the neonatal characteristics included gender, neonatal birth weight and blood group. One-way ANOVA, independent t-test, Pearson correlation, and single linear regression were used to determine the association between the factors and the haematopoietic contents of the UCB collected.

Results: Based on the NBC report, 39% (692) of the total UCB collected (1781) in 2014 met the acceptance criteria and stored. Low TNC and low UCB volume accounted for the highest reasons for UCB discard. Analysis of the maternal and neonatal factors showed that gestational age has a weak negative correlation with UCB volume ($r= -0.182$, $p=0.001$). While neonatal birth weight, is significant but showed weak correlation with CB volume ($r=0.174$, $p=0.001$) and TNC count/unit ($r=0.116$, $p=0.034$). Haematopoietic contents are all interrelated; the UCB volume with TNC count/unit ($r=0.594$, $p<0.001$), UCB volume with CD34+/unit ($r=0.364$, $p<0.001$) and UCB volume with CFU ($r=0.177$, $p< 0.015$).

Conclusions: This study concluded that maternal and neonatal factors were associated with the UCB haematopoietic contents. The factors identified were gestational age of the mother and new born weight which had significant association with the UCB volume and TNC count.

Keywords: Umbilical cord blood, donation, cord blood banking, maternal and neonatal factors

CHAPTER 1

INTRODUCTION

1.1 Overview

1.1.1 Introduction

Haematopoietic stem cell transplantation (HSCT) has become a standard treatment for many haematological malignancies (Little and Storb, 2002) and certain non malignant conditions (Harris 2008). It involves the infusion of haematopoietic stem cells (HSC) with the intention to restore and replace the haematopoietic system (Sureda *et al.,* 2015; Hatzimichael and Tuthill, 2010). The sources of HSC are either harvested from the patient themselves, known as autologous HSCT, or from matched healthy donors, such as in allogeneic HSCT (Henig and Zuckerman, 2014).

However, for many patients requiring allogeneic HSCT, fully compatible human leucocyte antigen (HLA) matched donors cannot be found in a timely manner (Kurtzberg *et al*., 2008). In this situation, umbilical cord blood (UCB) serves as a good alternative source of HSCs for transplantation (Little and Storb, 2002) as it is still possible to be performed despite the HLA mismatch (Gluckman and Rocha, 2009).

The first successful allogeneic UCB transplant in 1988 (Gluckman 2000), has paved way to currently more than 30,000 umbilical cord blood transplantation (UCBT) performed worldwide (Ballen *et al*., 2013). There were promising results with paediatric cases, yet the initial experiences of UCB with adults were associated with high

mortality rate (Ballen *et al.,* 2013). However, advancements have been made in UCBT for adult, which include improved patient selection, better supportive care, and transplantation with higher infused cell dose have improved the chance of survival (Ballen *et al.,* 2013). Results from several studies have revealed the comparable outcome of UCBT in adults compared to fully matched bone marrow (BM) transplant. This further support the use UCB as an alternative stem cell source for adult patients lacking HLA matched BM donor (Gluckman and Rocha, 2009).

1.1.2 Challenges in UCBT

There are many challenges faced by the UCB banks apart from high cost, donor recruitment and funding. Getting quality UCB for storage is one that is difficult to overcome. Despite promising results, there is significant delayed engraftment implicated in UCBT (Smith and Wagner, 2009). The main factors which influence engraftment are the cell dose of total nucleated cells (TNC) (Danby and Rocha, 2014), CD34+ cell concentration (Jaime-Perez *et al.,* 2011) and the degree of HLA matching (Danby and Rocha, 2014). UCB unit which contains high TNC count may yield more stem cells, and subsequently provide greater probability of engraftment (Patterson *et al.,* 2015).

The cord blood units collected by the UCB bank which meets the minimum cut off volume and TNC count set would be processed and stored. The volume of UCB correlates well with TNC count and CD34+ cell number (Allan *et al.,* 2013), hence it is the first criteria for storage by providing a quick estimation of UCB with bankable qualities.

However, one of the problems faced by the public UCB bank is to collect the cord blood units with enough cell dose of the TNC (Omori *et al.,* 2008). Various reports showed that more than 50% of the UCB collection were discarded and were not subjected to further processing due to this problem (Wang *et al*., 2014; Volpe *et al.,* 2011). This causes significant amount of wastage, financially as well as manpower.

This problem contributes to the UCB bank's challenge to maintain financial sustainability (Magalon *et al.,* 2015), as there is high cost implication involved (Sirchia *et al*., 1999; Petrini 2014). Thus, improvement in cord blood banking is needed in order to be more cost effective which include improving the collection performance as well as optimising the donor selection (Magalon *et al.,* 2015).

1.2 Justification and benefits

National Blood Centre (NBC), store more than 7500 units of UCB, which makes it the largest public UCB bank in Malaysia. The UCB collected are primarily for transplantation purpose. Thus, it is of utmost importance to collect and preserve the UCB units of the best quality to ensure the desirable outcome in transplant is achieved. This is dependent on the number of haematopoietic stem cells available in the UCB which is reflected in the number of TNC count, CD34+ cells and colony forming units (CFU).The acceptance criteria for bankable cord blood in NBC are UCB volume and TNC count, based on international standards. It is also in line with clinicians' preference of using the HSC source with the higher TNC count that directs the UCB collection to only preserve those with high likelihood to be used.

This has resulted in a high rate of rejection as revealed in the 2014 NBC statistics, more than 50% of the total UCB collected did not fulfill the minimum required criteria needed for banking. The rejections were primarily due to low TNC count as well as UCB volume. Thus, it would be beneficial to explore the selection and collection process before the UCB is processed to improve rate of acceptance.

Since the UCB are collected from healthy pregnant mothers, it would be best to identify whether the donor factors which include the maternal and neonatal factors would influence the quality of the UCB. This has been suggested by a previous study, which is to optimise donor selection in order for the UCB bank to be more cost effective (Magalon *et al*., 2015).

Thus, this study aims to analyse the maternal and neonatal factors and its influence on the quality of banked UCB collection in NBC. Therefore, the impact of various maternal and neonatal factors on the haematopoietic contents of the collected UCB units could be evaluated. This information will be utilised to facilitate and optimise the selection of the cord blood donor that would produce UCB that meet the required criteria of having the bankable and better quality UCB content. From this study, it is hoped that there will be improvement in selection of pregnant mothers that can potentially provide quality UCB to be collected and processed, thus creating a more efficient cord blood banking programme. The improvement in efficiency would also contribute toward cost saving. This study would also provide the baseline database of the UCB donors that would be of importance for future research as well as the expansion of the national cord blood collection program

CHAPTER 2

OBJECTIVES

2.1 General Objective

To determine the maternal and neonatal factors associated with haematopoietic contents among successfully banked and cryopreserved UCB blood units from January until December 2014 in NBC.

2.2 Specific objectives

- To determine the proportion of stored UCB units among donors in NBC.
- To describe the demographic factors among stored UCB donors from January until December 2014 in NBC.
- To determine the association of maternal and neonatal factors with the haematopoietic contents of stored UCB units among UCB donors in NBC.
- To determine the association between:
    - UCB volume unit with TNC count, CD34+cell concentration and CFU.
    - TNC count with CD34+ cell concentration and CFU.

2.3 Hypothesis

- The maternal and neonatal factors are significantly associated with haematopoietic contents of UCB.

2.4 Conceptual Framework

There are a few factors that may influence the haematopoietic contents of UCB as shown in the figure below. In this current study, the effect of maternal factors (age, race, gravid status, blood group and gestational age) and neonatal factors (newborn weight, gender and blood group) on the haematopoietic contents of UCB were examined. The association among the haematopoietic contents of UCB is also analysed.

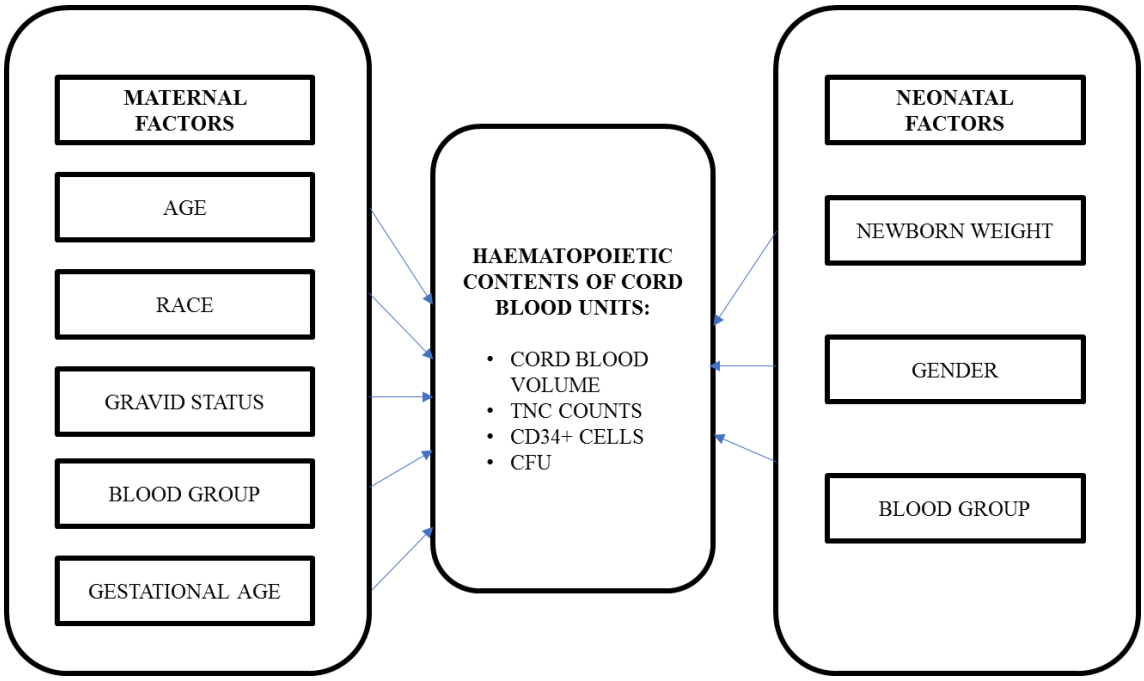

Figure 2.1: Analysis of the maternal factors and neonatal factors affecting the haematopoietic contents of UCB.

CHAPTER 3

LITERATURE REVIEW

3.1 Haematological diseases

Haematological diseases are disorders affecting haematopoietic cells which compromise the normal functions of red blood cells, white blood cells or platelets. Stem cell transplantation is capable of restoring normal blood homeostasis by replacing diseased cells with healthy haematopoietic stem cells.

3.2 Haematopoietic stem cell transplantation

Haematopoietic stem cells (HSCs) are self-renewing, multipotent cells which are isolated from the blood or bone marrow, and capable of giving rise to all haematopoietic cell types (Figure 3.1). These HSCs have full lineage differentiation capability to form more restricted oligopotent progenitors, the common lymphoid progenitor (CLP) and the common myeloid progenitor (CMP) **(**Seita and Weissman, 2010). The self-renewal capacity and differentiation ability is gradually lost as it matures into cells with specific function. The CLPs serve as the precursors of all lymphoid cells while CMPs are the precursors of all myeloid cells (Reya *et al*., 2001). Eventually, the CMPs will differentiate to granulocyte/macrophage progenitors (GMPs), which become committed into the granulocyte-monocyte lineage, while the MEPs, which produce erythroid and megakaryocyte cells. Whereas for the CLPs, they can give rise to B and T cell precursors and commit to the T and natural killer lineages (Doulatov *et al.*, 2012). These unique properties confer HSCs the ability to reconstitute bone marrow and restore haematopoiesis following transplantation (Seita and Weissman, 2010).

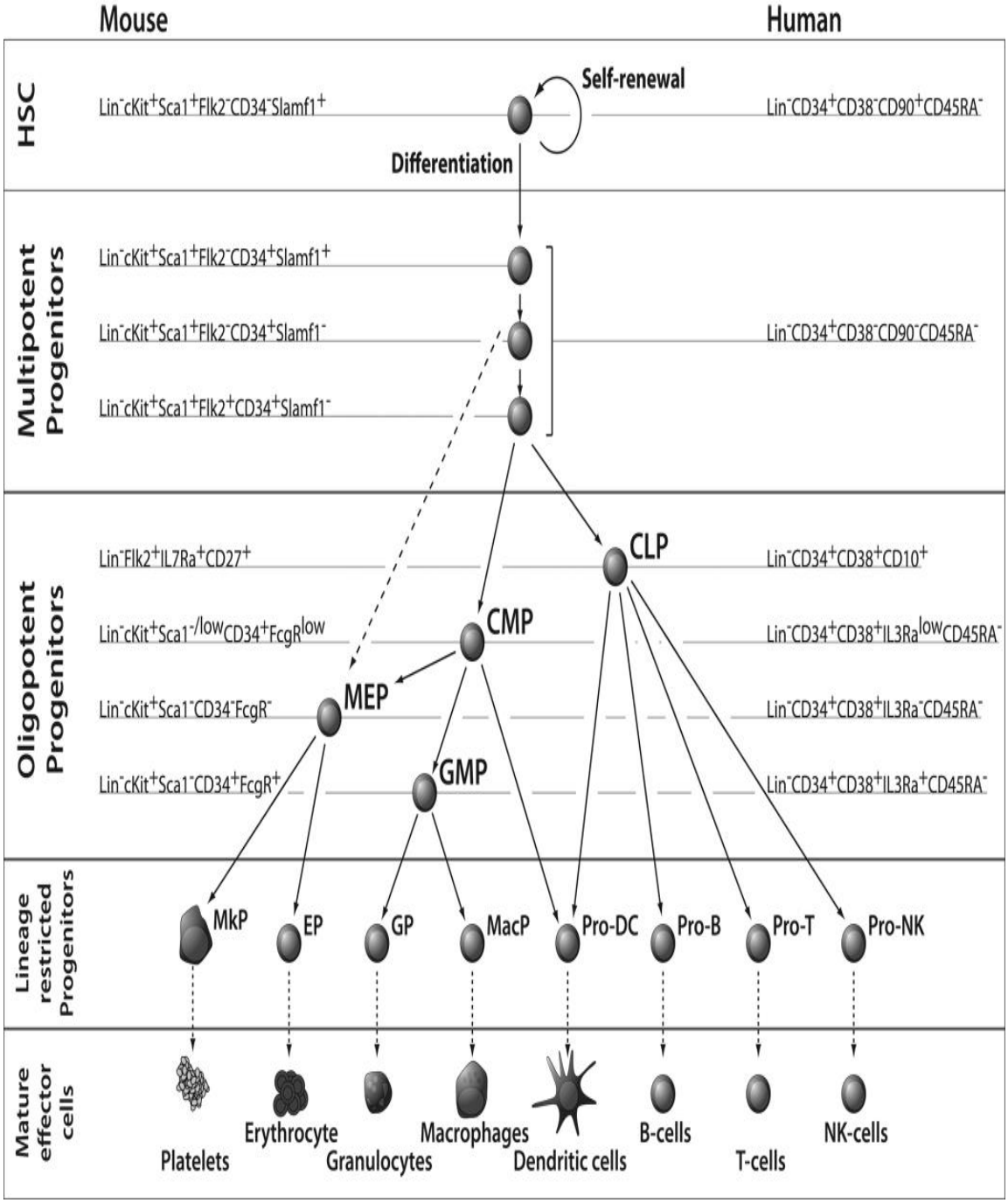

Figure 3.1: Model of the haematopoietic hierarchy (adapted from Seita and Weissman, 2010). HSC: Haematopoietic stem cell, CLP: Common lymphoid progenitor, CMP: Common myeloid progenitor, MEP: Megakaryocyte/Erythrocyte progenitor, GMP: Granulocyte/Macrophage progenitor, MkP: Megakaryocyte progenitor, EP: Erythrocyte progenitor, GP: Granulocyte progenitor, MacP: Macrophage progenitor, DC: Dendritic cell, NK: Natural killer, Lin: Lineage markers

Haematopoietic stem cell transplantation (HSCT) involves the infusion of HSCs with the intention to restore and replace the haematopoietic system and usually is preceded by a myeloablative preparative regimen (Sureda *et al.*, 2015; Hatzimichael and Tuthill, 2010). It has now become a standard treatment for many haematological malignancies (Little and Storb, 2002). It serves as the only proven modality of treatment and a hope of a long-term cure for certain non-malignant conditions (Harris 2008). Over the last two decades, the HSCT technology has undergone rapid expansion and constant development to extend its use in treating autoimmune and inherited metabolic disorders (Duncombe 1997; Henig and Zuckerman 2014).

There are two types of HSCT depending on the source of HSCs. In autologous HSCT, HSCs are harvested from the recipient themselves. For allogeneic HSCT, the source of stem cells is from healthy donors (Henig and Zuckerman, 2014). These donors may either be related or unrelated to the recipient, but possessing suitable cells for transplantation determined by the HLA (Ezzone and Schmit-Pokorny, 2007). Part of the protocol of HSCT involves the administration of high doses of chemotherapy for both malignant and non-malignant diseases (Goncalves *et al.*, 2009). After myelosuppression is induced, autologous or allogeneic HSCs are used as to restore the haematopoiesis (Seita and Weissman, 2010).

Table 3.1:Types of transplantation according to diseases (adapted from Henig and Zuckerman, 2014)

	Autologous Transplantation	**Allogeneic Transplantation**
Malignancies	Multiple myeloma Non-Hodgkin lymphoma Hodgkin disease Acute myeloid leukaemia Neuroblastoma Ovarian cancer Germ cell tumors Ovarian cancer Germ cell tumors	Acute myeloid leukaemia Acute lymphoblastic leukaemia Chronic myeloid leukaemia Myelodysplastic syndromes Myeloproliferative neoplasms Non-Hodgkin lymphoma
Non-malignant disorder	Autoimmune disease Amyloidosis	Aplastic anaemia Paroxysmal nocturnal haemoglobinuria Fanconi's anaemia Diamond-Blackfan anaemia Thalassaemia major Sickle cell anaemia Severe combined immunodeficiency Wiskott–Aldrich syndrome Inborn errors of metabolism Congenital neutropaenia syndromes

HSCT is indicated based on the patient's medical condition, treatment purpose, and the source of stem cells, be it allogeneic or autologous (Hatzimichael and Tuthill, 2010). Multiple myeloma, (50%), lymphomas (41%) and leukaemias (3%) are the most common diseases treated with autologous HSCs. As for acute myeloid leukaemia (AML) and acute lymphoblastic leukaemia (ALL), more than 50% of the cases involved allogeneic HSCT (Table 3.1) (Henig and Zuckerman, 2014).

Autologous HSCT involves the use of the patient's own marrow to reestablish haematopoietic cell function after the administration of high-dose chemotherapy. The major problem is that malignant cells, with their inherent resistance to chemotherapy,

might still survive and their reinfusion probably contributes to the high incidence of relapse observed after this form of therapy (Goncalves *et al*., 2009).

Allogeneic HSCT involves the administration of high doses of chemotherapy for both malignant and non-malignant diseases (Goncalves *et al*., 2009). The conditioning regimen aims to eradicate malignant cells, ineffective haematopoietic cells, and host immune cells, which may reject the donor cells (Hatzimichael and Tuthill, 2010). One advantage of allogeneic stem cells is its graft versus tumour effect that eradicates residual disease and prevent relapse (Hatzimichael and Tuthill, 2010).

Allogeneic HSCT has been proven to be an effective treatment for haematological malignancies (Nelson and Paulos, 2015) and thalassaemia (Isgro *et al.,* 2010). However, the main obstacle for allogeneic HSCT lies in the variation of normal human genomes which potentially induce immunogenic reaction causing graft rejection, graft versus host disease or graft versus tumour reaction after transplantation (Nowak 2008). These rejections are mainly mediated by HLA (Little and Storb, 2002) which is encoded by the major histocompatibility complex (MHC). The HLA match between a donor and recipient is determined by a genetic analysis performed prior to a transplant. Currently the standard of HLA matching is by doing HLA at A, B, C, DRB1 and DQB1 genetic loci (Nowak 2008).

The use of HSCT continues to expand and evolve with improving safety and efficacy to include wider indications. It has been proven to be the chosen treatment modality as it offers higher chance of cure and longer survival (Maziarz *et al*., 2014). Yet, there is still high morbidity associated with HSCT arising from the high dosage of

chemotherapy. This confers substantial drug toxicities and complications from prolonged immunodeficiency to the patients (Hatzimichael and Tuthill, 2010). However, advancement such as specific scoring systems have been developed reduces the risk of transplantation related mortality (Henig and Zuckerman, 2014).

3.3 Limitation of HSCT

The most ideal scenario for allogeneic transplantation is having a fully matched donor possessing HLA allele HLA-A, -B, -C, and -DRB1 compatible with the recipient (Eapen *et al.*, 2014). However, only one third of these patients were able to receive bone marrow HSC from HLA matched related donor (La Nasa *et al.*, 2002; Ballen *et al*., 2001). About 50 -70% of the patients in need of HSC transplant are still unable to find a suitable related or unrelated adult donor especially within a short time period in the United States. Despite 13 million of unrelated adult donor volunteers registered in the database of the National Marrow Donor Program (NMDP) and other international registries (Kurtzberg 2009), it still remains a challenge to find a suitable matched donor due to the time and cost incurred. This problem becomes more difficult in patients among ethnic minorities due to the limited donor availability (Kurtzberg 2009). The challenge for countries with diverse racial and ethnic backgrounds like in Malaysia is to include as many ethnic groups into the bone marrow registry as well as UCB bank.

The recommended alternate HSC sources for such patients include haploidentical related donors, matched unrelated donors (MUD), mismatched unrelated donor and umbilical cord blood (UCB). (Ballen *et al.*, 2013); (Fabricius and Ramanathan, 2016). Haploidentical donors share one similar HLA haplotype with the recipient, and they are

commonly the biological parents, children, full or half siblings as well as collaterally-related donors. This measure allows transplantation to be performed more promptly than the traditional transplantation with unrelated donor grafts. Yet, it will impose a risk of introducing triggering intense bidirectional alloreactivity and high incidences of graft rejection as well as graft-versus-host disease (GVHD) (Fabricius and Ramanathan, 2016). A study revealed that a single or two HLA mismatches (2 out of 8) could risk patients' survival, of which a single HLA mismatch could introduce 25% increase in the risk of mortality in comparison to a fully matched transplant (Anasetti *et al*., 2012).

3.4 UCB HSCs in HSCT

UCB is an ideal source of stem cells for allogeneic HSCT, and the cells commonly serve as an alternative source for patients who do not have matched HLA-compatible donors (Querol *et al.*, 2010). Cord blood HSCs comprise of progenitor cells that are capable to engraft and reconstitute the entire haematopoietic system in recipients (Querol *et al*., 2010).

The cord blood is obtained from consented healthy mothers during their third stage of labour, immediately or prior to placental expulsion (Solves *et al.*, 2005). UCB is collected either as ex utero or in utero method of collection. During ex utero collection, the UCB is collected after the delivery of placenta where the placenta will be placed on a sterile surgical tray. For in utero collection, the UCB is collected prior to the delivery of the placenta immediately after the umbilical cord is clamped. Both strategies employ gravitational method to drain UCB from umbilical vein into a sterile collection bag with

anticoagulant (Solves *et al.*, 2003). In NBC, UCB is collected using in utero method which has also been found to be a better approach (Solves *et al.*, 2003).

UCB offers several advantages such as the relative ease of procurement procedure, as it entails a safe procurement method with minimal of risk to donors (Wang *et al.*, 2014), in addition to reduced risk of disease transmission (Gluckman *et al.*, 2011). UCB also has a large donor pool, mostly made readily available within short dispatch time (Oran and Shpall, 2012). It is estimated that there are more than 500,000 units of UCB available for unrelated donor haematopoietic stem cell transplant (Oran and Shpall, 2012).

Furthermore, UCB are generally immunogenically naïve, and thus better serves as an alternative to those patients of diverse ethnic backgrounds, whom do not have a suitably matched, unrelated voluntary donor identified in the required time frame (Ballen *et al.*, 2013). Compared to bone marrow transplantation (BMT), of which a fully compatible HLA match is often required, UCBT can still be performed when the donor cells possess HLA disparities (Gluckman 2000; Gluckman and Rocha, 2009).

Primitive CD16-CD56++ natural killer (NK) cells, which possess significant proliferative and cytotoxic capacities, are also found in abundance in UCB. These cells can be further expanded using IL-12 or IL-15, and potentially promotes graft versus leukaemia (GVL) effect (Cohen and Nagler, 2004). Furthermore, the UCB derived HSCs were also found more primitive compared to those present in the bone marrow (BM) or peripheral blood (PB) (Hordyjewska *et al.*, 2015). The highly-proliferative

colony-forming cells are found in large quantity in UCB which exceeds eight times that of bone marrow (Nimgaonkar *et al*., 1995; Hordyjewska *et al.,* 2015). This confers UCB HSCs with a greater capability to repopulate the recipient's haematopoietic system.

3.5 Progress of UCBT

The first UCBT was performed in October 1988 to treat a patient with Fanconi Anaemia. Twelve years after the procedure, the patient had progressed well and had full donor haematopoietic reconstitution (Gluckman 2000). In 1993, 25 successful transplantations were performed using unrelated UCB in the New York, demonstrating its clinical feasibility (Ballen *et al*., 2013). Analysis between UCBT and BMT with the present HLA matching practice showed that HLA matched and one or two antigen HLA mismatched UCB can be transplanted to treat children with acute leukaemia. Better HLA matching outcome and higher cell dose were found to significantly decrease the risk of transplant related mortality (Eapen *et al.,* 2007).

Eurocord, a collaborative effort of public European cord blood banks and registries (http://www.eurocord.org/about-eurocord.php), compared the outcomes of matched unrelated BMT to HLA mismatched UCBT. They observed that UCBT are associated with delayed engraftment and lesser event of acute and chronic GVHD, yet relapse rate, overall survival, and leukaemia-free survival (LFS) were similar to matched unrelated donor (MUD) bone marrow (Ballen *et al.,* 2013).

Another study had compared fully HLA matched unrelated adult peripheral blood (PB) stem cell transplant to HLA mismatched UCB transplant for patients with myelodysplastic syndromes (MDS) from 2005 to 2011(Ruggeri *et al.*, 2013). The results revealed that a fully HLA matched unrelated matched PB donor, a 9/10 HLA matched PB donor or mismatched UCB gave similar outcomes. Therefore, for MDS patients without a fully HLA matched unrelated donor, a graft with 9/10 HLA matched or a HLA mismatched UCB are both alternative options for transplantation (Ruggeri *et al.*, 2013).

A further study by Eurocord, comparing adults with acute leukaemia receiving either a full 6/6 HLA matched unrelated BMT and a mismatched UCB transplant showed promising results. UCBT had a similar LFS to BMT despite a delay in engraftment. Moreover, Centre for International Blood and Marrow Transplant Research (CIBMTR) demonstrated that UCB transplant in adults with malignancies have produced the same LFS as compared to one HLA antigen mismatched BMT (Gluckman and Rocha, 2009).

Several comparative studies and meta-analysis concluded that the outcome of UCB transplant in adults was comparable to fully matched bone marrow (BM) transplant. Thus, UCB is an alternative source of allogeneic stem cell for adult patients lacking HLA matched BM donor (Gluckman and Rocha, 2009).

3.6 UCB content and therapeutic

Haematopoietic engraftment refers to the period post transplantation where the blood parameters have recovered and transfusion support is not required anymore (Allan *et al.*, 2013). It is one of the parameters in determining the successful clinical outcomes of

transplantation (Ratajczak & Suszynska, 2016). There was significant delay of haematopoietic engraftment for UCB graft compared with other sources of HSC, causing the period of aplasia post transplantation to be to longer as well. This result in UCBT has substantial risk of infection-related morbidity and mortality (Danby and Rocha, 2014).

The main factors influencing engraftment are the cell dose of TNCs (Danby and Rocha, 2014), CD34+ cells concentration (Jaime-Perez *et al.*, 2011) and the degree of HLA matching (Danby and Rocha, 2014). The cell dose refers to the TNC count per recipient body weight in kilogram, and it is proportionate to the haematopoietic potential of UCB collections (Migliaccio *et al*., 2000). The cell dose in UCB unit is one of the most critical determinants of therapeutic efficacy, transplantation related mortality as well as overall survival after UCBT (Scaradavou *et al*., 2013).

TNC refers to the number of nucleated cells that can be found in the circulation for at least a period of their life (Walker *et al.*, 1990). A higher TNC count reflects a higher proportion of proliferative stem cells, which offer greater probability of engraftment (Patterson *et al.,* 2015). UCBT trials in adult have showed that higher UCB derived TNC count was associated in earlier neutrophil recovery, while greater event free survival was found to be associated with greater CD34+ cell dose (1.2×10^5 cells/kg) (Oran and Shpall, 2012). However, the major limitation in UCBT is the infrequent availability of UCB units with the minimum acceptable pre-cryopreservation cell dose of TNC count of 2.5×10^7/kg patient body weight (Scaradavou *et al.,* 2013).

Other informative indices of haematopoietic potential include enumeration of CD34+ cell and colony forming progenitor cells or CFU (Migliaccio *et al.,* 2000). CD34 is a transmembrane phosphoglycoprotein and it is regarded as a marker of HSC and haematopoietic progenitor cells (Sidney *et al.,* 2014). It has been shown that CD34+ cell content influence engraftment and survival after unrelated UCBT (Jaime-Perez *et al.,* 2011). CFU assays are in vitro functional assays to identify haematopoietic progenitors (Frisch and Calvi, 2014). Colonies of maturing cells are produced as the haematopoietic progenitor cells proliferate and differentiate when cultured using methylcellulose-based medium enriched in a suitable environment (Wognum *et al.,* 2012). This assay is important in evaluation of functional capacity of HSCs after thawing the UCB product, however due to the variation of colony setup and counting between centres, there is difficulty in establishing a generalized CFU dose for transplantation purposes (Hough *et al*., 2016).

A recommendation for a standard UK approach to incorporate UCB into clinical transplantation practice was accomplished after a meeting between senior transplant physician, UK cord blood banks and scientists from across UK. Through these recommendations, it was aimed to focus on UCB unit selection, donor selection strategy and conditioning regimens. According to the consensus, TNC is regarded as the primary cell dose criterion. Despite the fact that the CD34+ cell count may be a better marker of cell content, there are historical differences in CD34 quantification methodologies and availability which makes interbank comparison difficult. Another good marker of HSCs proliferative potential is the CFU in the UCB grafts. However, not many studies have documented its' association with engraftment or survival and not all collection centres are able to perform this test due to various reasons (Hough *et al.,* 2016). Based on these

recommendations, TNC is considered as the primary criterion pertaining to UCB selection for transplantation in which it is also one of the criteria for banking. Thus it is important to see the association of TNC with the other marker of cell content such as CD34+ and CFU.

There have also been numerous strategies to optimise cell number and improve the rate of neutrophil, platelet recovery, and engraftment after UCBT. These strategies include improving cord blood collection and processing, enhanced HLA matching, improving homing and ex vivo expansion of UCB. Usage with other grafts includes double UCB grafts, co transplantation with third party donor and infusing with accessory cells (Danby and Rocha, 2014).

Use of double UCB grafts by co infusing two partially HLA-matched cord blood units especially in adult patients has been one of the commonly employed strategies. According to the European registry (Eurocord), since 2005 the usage of double UCB grafts has even surpassed the number of adult patients transplanted with single cord blood units (Sideri *et al.*, 2011). The transplant outcome with regards to the disease free survival, neutrophil recovery, transplantation related death, relapse, infection, immunologic reconstitution and acute GVHD were found to be similar to the use of the single UCB graft. Furthermore, double UCB grafts were also compared to other stem cell sources, which include matched sibling donor and matched unrelated donor which showed similar outcome for leukaemia free survival at 5 years, transplanted related mortality and relapse related death. This method has also been adopted by NBC in which all the adult patients utilized double UCB grafts (National Stem Cell Coordinating Centre, unpublished data).

3.7 UCB banking

The progression in UCBT has attracted huge interest in establishing cord blood banking services worldwide (Gluckman 2009). Cord blood banks are either intended for the public use or personal purposes. The first public UCB bank was established in 1993 at New York, United States. It has since grown to more than 50 UCB banks worldwide, with over 600,000 UCB units stored for transplantation (Ballen *et al*., 2013; Liu *et al.*, 2012). With the growth of numerous UCB banks, there was a need to establish good practices in regard to umbilical cord blood storage. The Netcord group was founded in 1998 to accomplish this task. This organization will facilitate donor search, improve the quality of grafts, and standardize excellence criteria on an international scale and more importantly, to establish procedures for banking accreditation (Gluckman 2009).

Public cord blood banks need to have an optimal inventory in order to have UCB units with a good HLA match with a patient, which is dependent on the size of the population and presence of ethnic minorities (Querol *et al.,* 2009). Study showed that the likelihood of getting UCB with good HLA match would be greater with an inventory of 1 or 2 units for a population of 1,000 people (Petrini 2014).

Each UCB banking programme establishes its own UCB acceptance criteria prior to cryopreservation. Most cord blood centres require a minimum limit of UCB volume and TNC count as the main selection factors for cryopreservation (Jaime-Perez *et al.,* 2011; Patterson *et al.,* 2015) to facilitate the efficient UCB units for clinical use (Omori *et al.,* 2012). Yet many countries have reported a high discard rate by adhering to this requirement, which contributes to significant amount of wastage in terms of finance and

manpower. A higher discard rate would raise the cost in attaining each UCB unit (Petrini 2014)

This causes a shortfall in UCB banking as many UCB banks have already found it difficult to be sustaining financially (Magalon *et al.*, 2015). There is high cost involved in the present standard of UCB banking which include the high personnel cost, collection, processing and storage (Omori *et al.,* 2012). An economic study by the Spanish Plan Nacional de Sangre de Cordón Umbilical found the minimum cost for each UCB unit is €1,300 ($1,760) for collection and processing while €40/year ($65/year) for storage, based on a 50% discard rate. The cost is increased to €1,600 ($2,170) if the discard rate increases to 65% and reaches almost €1,900 ($2,580) as the discard approaches 80% (Petrini 2014)

New strategies and improvising are needed in order to minimize operating costs. Among the recommendations include improving collection performance of the UCB units as well as optimizing donor selection based on maternal and infant characteristics which influence UCB quality (Magalon *et al*., 2015).

The NBC also faces the same challenge in order to sustain financially with a discard rate almost as high as other countries. Currently, it is estimated a minimum cost of RM 3300 per each UCB stored in NBC rate (unpublished data, Cord blood unit) which include the cost of collection, processing and storage for one year, excluding the cost of manpower as well as utilities.

It would be beneficial to the NBC to implement the proposed recommended strategies in order to become more cost efficient. Thus, optimizing the donor selection would assist in improvement in UCB collection. By analyzing the donor characteristics with the haematopoietic contents of the UCB, this would then help in the selection of donors who are most likely to have adequate parameters for banking

3.8 UCBT in Malaysia

The first public cord blood bank was established by the NBC in 2001, which is located in Kuala Lumpur, Malaysia. This was established following the need among paediatric patients based on disease population studies. The number of children are diagnosed with leukaemia is approximately to be 3000 each year in Malaysia. Currently there are 26,000 donors for matched unrelated donors under the Malaysian Stem Cell Registry whereas it is estimated at least 40,000 donors to have a good match for the purpose of allogeneic transplantation (Malaysian Stem Cell Registry, 2013 http://www.imr.gov.my).

The expansion of the public cord blood bank's service by the Ministry of Health was in line with the anticipated demand (National standards for cord blood banking and transplantation, 2008). Four hospitals were designated as the site of umbilical cord blood collection in Klang Valley. General Hospital Kuala Lumpur has started the cord blood collection since 2001, followed by Hospital Selayang (2011), Hospital Serdang (2012) and Hospital Ampang (2013). These UCB collections are processed and subsequently stored in NBC. In April 2010, Hospital Sultanah Bahiyah in Kedah was established to cater for the northern population.

From its establishment until 2014, over 16,677 units of cord blood units have been collected for cord blood transplant but to meet the NCB criteria, only 7,505 UCB units were subsequently processed and stored. In this study which analyzed the UCB collection in 2014, it was found that from 1781 UCB collected, 58.3% (1045) were discarded (unpublished data, Cord blood unit).

The acceptance criteria for banking in NBC are UCB volume >50mls and TNC count more than $6X10^8$ cells. Those UCB which meet the minimum requirement will then be processed for enumeration of CD34+ cells and CFU colonies. The NCB standards were primarily adapted from the NETCORD-FACT international standards (National Standards for Cord Blood Banking and Transplantation, 2008).

Table 3.2: Requirements prior to processing

Processing requirement:
UCB volume >50mls TNC count >$6x10^8$ Complete documentation Temperature storage 22-24 Processed within 48hours after collection

However, the donor criteria for donations among pregnant mothers are very general (Table 3.3). Currently, the present guideline only focuses on issue of safety of the donation and does not help with the selection of likelihood of having bankable UCB units. Thus, by analysing the donor characteristics to see if there are any associations or influence towards the quality of the UCB perhaps would enable the UCB collectors to predict the donors with higher chance of having better quality UCB units.

Table 3.3: UCB donor criteria in NBC (Refer Appendix 1)

Donor criteria:
Age >18 years old Malaysian Married Singleton pregnancy Gestational age >36 weeks Antenatal screening: negative for HIV, Hepatitis B, C and Syphilis No high risk behaviour No chronic illness or regular medication No maternal or paternal history of genetic disease

Cord blood banking is costly to which involve many processes, including collection, processing and storage of the store UCB unit. Currently, it is estimated a minimum cost of RM 3300 per each UCB, which include the cost of collection, processing and storage 1 year, excluding cost of manpower as well stored in NBC with the current discard rate (unpublished data, Cord blood unit).

Any request for allogeneic stem cell made to the National Stem Cell Coordinating Centre (NSCCC) a search will be made to the National Bone Marrow Registry as well as to the cord blood unit at NBC. The total request for UCB searches have reached more than 355 from all over the country, but there have only been 5 cases of UCBT performed using UCB units from NBC (NSCCC census, NBC). These cases involved a paediatric patient with aplastic anaemia (2009); adult case of AML (2010); adult case of relapse ALL (2013); paediatric patient with Fanconi's anaemia (2013) and most recently an adult patient with ALL (2016) (unpublished data, Cord blood unit).