

Factors Influencing The Provision And Clinical Usage Of Umbilical Cord Blood Units As A Graft Source In Unrelated Haematopoietic Stem Cell Transplantation

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**A Thesis Submitted to the Cancer Institute, University College London (UCL),
for the Degree of Doctor of Medicine (Research)**

Author's Declaration

I, Olga Nikolajeva, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

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Date

Abstract

Hematopoietic stem cell transplantation (HSCT) is currently used as a potential curative therapy for several malignant and non-malignant disorders. In recent years our understanding of complexity of immunogenetics of HSCT has led to new indications for the procedure. Therefore the demand for unrelated hematopoietic stem cell (HSC) donors keeps rising. HLA matching remains the main determining factor when searching for an HSC donor. An HLA-identical sibling still remains the most suitable donor, but only around 30% of patients (requiring HSCT) have this option. Others require a search for an alternative donor. Because the HLA system is highly polymorphic, the chance of finding a suitable matched unrelated donor is limited. When bone marrow or peripheral blood are used as source of HSC, stringent HLA-matching is required to lower the risk of transplant-related complications. The donor pool is especially limited for patients with diverse racial and ethnic backgrounds because such ethnicities are underrepresented on current worldwide donor registries (Barker, Byam, et al., 2010). To overcome this problem, unrelated umbilical cord blood units (UCB) can be selected as readily available alternatives for patients who lack both HLA-matched siblings and suitable unrelated donors.

This thesis provides an insight into the contemporary factors influencing selection of unrelated umbilical cord blood (UCB) for transplantation.

Studies in this thesis provide a detailed analysis of current UCB selection practice and explore new factors that can help to develop a patient tailored approach to the UCB selection process. Work is based on the understanding of a tri-dimensional donor-recipient interaction process in the context of UCBT, which involves not only direct donor-recipient interaction, but also involves foeto-maternal exchange of information.

My key finding was that maternal characteristics (i.e. previous pregnancy and exposure to infections) influence quality characteristics of UCB and potentially have an impact on transplant outcomes.

Based on my study findings, a new project has been initiated. It will study a larger cohort of patients and investigate the impact of birth order and previous child's sex on UCBT outcome.

Table of Contents

ABSTRACT	3
TABLE OF CONTENTS	5
ACKNOWLEDGEMENTS	9
MANUSCRIPTS IN PREPARATION	12
PEER-REVIEWED PRESENTATIONS	13
LIST OF FIGURES	14
TABLE OF TABLES	16
GLOSSARY	18
THESIS OVERVIEW	25
CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW	28
1.1. HISTORY AND CONTEXT.....	28
1.1.1. <i>Umbilical cord blood (UCB) as a source of stem cell procurement</i>	28
1.2. UCB BANKING.....	35
1.3. FACTORS KNOWN TO INFLUENCE UMBILICAL CORD BLOOD TRANSPLANT OUTCOMES	37
1.3.1. <i>Introduction</i>	37
1.3.2. <i>TNC and UCB Selection</i>	38
1.3.3. <i>UCB selection and CD34+ cell count</i>	45
1.3.4. <i>UCB graft selection and non-inherited maternal antigens (NIMA) and inherited paternal antigens (IPA)</i>	47
1.3.5. <i>UCB selection and Killer-immunoglobulin-like receptor (KIR) ligands</i>	50
1.4. OTHER FACTORS.....	51
1.4.1. <i>Functional assays of UCB quality</i>	51
1.4.2. <i>Cytomegalovirus</i>	52
1.4.3. <i>UCB selection, donor-specific HLA allo-antibodies and ABO mismatch</i>	55

1.4.4.	<i>UCB unit cryopreservation time and transplant outcomes</i>	56
1.5.	THE ROLE OF HLA IN UCBT	58
1.5.1.	<i>Introduction</i>	58
1.5.2.	<i>UCB-patient HLA-matching and the impact on transplant outcome</i>	58
1.6.	IMMUNE RECONSTITUTION POST UCBT	61
1.7.	CONCLUSION.....	65
CHAPTER 2. DELIVERY MODE INFLUENCE ON THE VOLUME AND TOTAL NUCLEATED CELL		
COUNT OF THE UCB		
67		
2.1.	INTRODUCTION	67
2.2.	MATERIALS AND METHODS	69
2.3.	RESULTS.....	71
2.3.1.	<i>Collection mode</i>	71
2.3.2.	<i>UCB volume</i>	71
2.3.3.	<i>UCB TNC</i>	71
2.3.4.	<i>Mode of the delivery and UCBs stored for clinical use</i>	72
2.4.	DISCUSSION.....	74
CHAPTER 3. DONOR-RECIPIENT ALLELE-LEVEL HLA MATCHING OF UNRELATED CORD		
BLOOD UNITS.....		
78		
3.1.	INTRODUCTION	78
3.2.	MATERIALS AND METHODS	81
3.3.	RESULTS.....	81
3.3.1.	<i>Characteristics of recipients</i>	81
3.3.2.	<i>Extent of UCB-recipient pair HLA mismatch at low/intermediate resolution</i>	83
3.3.3.	<i>Extent of UCB-recipient HLA mismatch at high resolution</i>	84
3.3.4.	<i>UCB-recipient HLA allelic match and TNC dose</i>	86
3.4.	DISCUSSION.....	86
CHAPTER 4. RELATIONSHIP BETWEEN DONOR MOTHERS PREVIOUS EXPOSURE TO THE		
VIRAL INFECTIONS AND UCBS CELL COUNT.....		
89		

4.1.	INTRODUCTION.....	89
4.2.	MATERIALS AND METHODS	94
4.2.1.	<i>Statistical analysis</i>	96
4.3.	RESULTS	97
4.3.1.	<i>Frequency of positive IgG class antibodies for the CMV, EBV and Toxoplasma gondii</i>	97
4.3.2.	<i>Cell count in the UCBs from both CBB</i>	99
4.3.3.	<i>Relationship between TNC, CD34+ cells, mononuclear cells and lymphocytes count and maternal CMV serological status</i>	99
4.3.3.1.	Anthony Nolan Cell Therapy Centre	100
4.3.3.2.	Barcelona's CBB.....	100
4.3.4.	<i>Relationship between TNC, CD34+ cells, mononuclear cell and lymphocytes count and maternal EBV serological status</i>	101
4.3.4.1.	Anthony Nolan Cell Therapy centre.....	101
4.3.4.2.	Barcelona's CBB.....	102
4.3.5.	<i>Relationship between TNC, CD34+ cells, mononuclear cell and lymphocytes count and maternal Toxoplasma gondii serological status</i>	103
4.3.5.1.	Anthony Nolan Cell Therapy centre.....	103
4.3.5.2.	Barcelona's CBB.....	104
4.4.	DISCUSSION.....	105
CHAPTER 5. MATERIALS AND METHODS		109
5.1.	INTRODUCTION.....	109
5.2.	STUDY POPULATION	109
5.3.	DATA COLLECTION.....	110
5.4.	STUDY END-POINTS AND STATISTICAL ANALYSIS	110
CHAPTER 6. IMPACT OF MATERNAL CMV SEROLOGICAL STATUS ON UCBT OUTCOME IN RECIPIENTS WITH ACUTE LEUKAEMIA.....		115
6.1.	INTRODUCTION.....	115
6.2.	STUDY POPULATION	118
6.3.	MATERIALS AND METHODS	118

Table of Contents

6.4.	RESULTS.....	119
6.4.1.	<i>Patients, disease and graft characteristics</i>	<i>119</i>
6.4.2.	<i>Neutrophil engraftment.....</i>	<i>121</i>
6.4.3.	<i>Acute and chronic Graft-versus-Host Disease (GvHD).....</i>	<i>125</i>
6.4.4.	<i>Relapse.....</i>	<i>127</i>
6.4.5.	<i>Non-relapse mortality.....</i>	<i>130</i>
6.4.6.	<i>Overall survival and disease-free free survival.....</i>	<i>135</i>
6.5.	DISCUSSION.....	145
CHAPTER 7.	CONCLUSIONS.....	159
7.1.	SUMMARY.....	159
7.2.	CHALLENGES.....	162
7.3.	FUTURE PROJECTS.....	163
7.3.1.	<i>Immunophenotyping of cord blood units collected from CMV seropositive mothers.....</i>	<i>163</i>
7.3.2.	<i>A study of the influence of previous donor pregnancies on the outcome of unrelated cord blood transplants for malignant disorders.....</i>	<i>164</i>
7.4.	CONCLUSION.....	165
REFERENCES.....		167
APPENDIX – SUPPLEMENTAL TABLES AND FIGURES.....		181

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Nikolajeva O. et al. Patient-umbilical cord blood four loci high resolution matching may significantly change cord blood unit selection.

List of Figures

Figure 1-1: The number of banked Cord Blood Units Worldwide (“WMDA-Home”)	36
Figure 1-2: Number of UCBT in adults per year reported to Eurocord (Annalisa Ruggeri, 2016).....	40
Figure 1-3: IPA and NIMA in UCB (Milano, Lee Nelson, & Delaney, 2013)	48
Figure 1-4 : Diagram of the study on the incidence of CMV infection post UCBT	54
Figure 2-1 : Clinical conversion rates with breakdown of collection type	73
Figure 3-1: Distribution of low/intermediate level umbilical cord blood unit-recipient HLA match	83
Figure 3-2: Comparison of patient/UCB 4-loci allelic level matching and “classic” level of matching.....	86
Figure 4-1: CMV-specific CD8+ T-cells phenotypes. La Rosa, C., & Diamond, D. J. (2012).....	91
Figure 4-2 : Study groups: schematic representation	95
Figure 4-3 : Frequency of maternal positive serologic status for CMV, EBV and Toxoplasma gondii in both CBBs.....	98
Figure 4-4: Association between maternal anti-CMV IgG positivity and UCBs TNC and lymphocyte count	100
Figure 4-5 : Association between maternal anti-CMV IgG positivity and UCBs TNC and lymphocyte count	101
Figure 4-6: Association between maternal anti-EBV IgG positivity and lymphocyte and mononuclear cell count.....	103

Figure 6-1: Probability of neutrophil engraftment at Day 60. (A): all patients. (B): effect of collected TNC dose. (C): effect of collected CD34+ cell dose...	125
Figure 6-2: Cumulative incidence of 5-y relapse after unrelated UCBT for acute leukaemia. (A): for all patients. (B): effect of disease stage before UCBT. (C): effect of conditioning regimen. (D): effect of serotherapy (ATG).....	129
Figure 6-3: Cumulative incidence of 5-y NRM after unrelated UCBT for acute leukaemia. (A): for all patients. (B): effect of maternal previous pregnancies. (C):: effect of number of maternal prior pregnancies.....	134
Figure 6-4. Influence of maternal numbers of previous pregnancies to 5-year OS.	137
Figure 6-5: Influence of maternal CMV status on 5-year OS.	138
Figure 6-6: The probability of 5-year DFS according to disease stage	139
Figure 6-7. Influence of maternal previous pregnancies to 5-year DFS.....	141
Figure 6-8: Influence of maternal number of previous pregnancies to 5-year DFS.	142
Figure S- 1: Study Proposal of Examining the Association between Maternal CMV Serostatus and UCB Cell Count	181
Figure S- 2: Study Proposal for the Project on Donor Recipient Allele-level HLA Matching of Unrelated UCB	184
Figure S- 3: Study Proposal for the Eurocord Study	186
Figure S- 4: Letter sent to CBB giving synopsis of the study and requesting approval	191

Table of Tables

Table 1-1: Results of comparative studies on BMT, PBSCT and UCBT in adults and children.....	30
Table 1-2 : Advantages and limitations of umbilical cord blood unit in comparison with bone marrow or peripheral blood stem cells.....	33
Table 1-3 : Overview of the studies that evaluated influence of TNC dose on UCBT outcomes after single UCB infusion.....	40
Table 1-4 : Overview of the studies exploring UCB expansion	43
Table 2-1: Maternal and obstetric factors influence on the quality of UCB.	68
Table 2-2 : Collected UCBs parameters (volume and TNC)	72
Table 3-1 : Patients Characteristics	82
<i>Table 4-1 : Summary of UCBs cell count.....</i>	<i>99</i>
Table 4-2 : Association between maternal anti-EBV IgG positive AB and UCBs cell content	102
Table 4-3: Association between maternal anti-EBV IgG positive AB and UCBs cell content	103
Table 4-4: Association between maternal anti-Toxoplasma gondii IgG positive AB and UCBs cell content	104
Table 5-1: Definitions of Outcomes.....	111
Table 6-1: Characteristics of 1177 study patients and UCB grafts.....	120
Table 6-2: Univariate analysis of neutrophil engraftment for 1177 patients with Acute Leukaemia.....	122
Table 6-3: Factors associated with Grade II-IV acute GvHD in univariate and multivariate analysis.	126

Table 6-4: Univariate analysis of main factors associated with 5-year NRM...	130
Table 6-5: Univariate analysis of factors associated with 5-year OS after UCBT for 1177 patients with Acute Leukaemia.....	135
Table 6-6: Univariate analyses of 5-year DFS after UCBT for 1177 patients with Acute Leukaemia.....	140
Table 6-7: Multivariate analysis of risk factors for each main outcome after UCBT for 1177 children and adults with AL.....	143
Table S-1: Example of a Spreadsheet used for the Study Examining the Association between Maternal CMV Serostatus and UCB Cell Count ...	183
Table S-2: Example of a Spreadsheet used for the Study on Donor Recipient Allele-level HLA Matching of Unrelated UCB	185
Table S-3: Example of a Spreadsheet used for the Collection of Information on UCBs from CBBs participating in the Eurocord study	193
Table S-4: Example of a Spreadsheet on Patients, Graft and Transplant Characteristics and UCBT Outcome Received from the Eurocord Electronic Database – Part 1.....	194
Table S-5: Example of a Spreadsheet on Patients, Graft and Transplant Characteristics and UCBT Outcome Received from the Eurocord Electronic Database – Part 2.....	195
Table S-6: Example of a Spreadsheet on Patients, Graft and Transplant Characteristics and UCBT Outcome Received from the Eurocord Electronic Database – Part 3.....	196

Glossary

Term	Definition
Allele	One of a number of different forms of a particular genetic locus. The HLA genes together have several thousand different alleles.
Allo-HSCT	See 'Allogeneic hematopoietic stem cell transplant (allo-HSCT).
Allogeneic hematopoietic stem cell transplant (allo-HSCT)	In humans, allo-HSCT refers to the use of donated HSC from another individual for transplantation. Examples of HSC sources for allo-HSCT include: syngeneic (twin) donor, HLA-identical sibling donor, haplo-identical related donor, unrelated adult donor, and cord blood unit.
Antigen	A substance that is bound by a particular antibody. In terms of HLA, and antigen is the protein product of a particular HLA gene to which a specific antibody binds.
BMDW	Bone Marrow Donors Worldwide. A global collaborative collation system designed to provide a searchable interface for unrelated donors listed by participating registries.
BSBMT	The British Society of Blood and Marrow Transplantation
CBU	See 'Cord Blood Unit'
CBWG	Cord Blood Working Group was established in 2008 to provide UK transplant centers with recent recommendations on selection of cord blood units for the HSCT.

CFU	Colony-forming unit.
Chimerism	In allo-HSCT, chimerism refers to the relative proportion of donor and recipient cells (of hematopoietic origin) found in the recipient blood or bone marrow following transplant. Chimerism studies are particularly necessary following reduced-intensity conditioned allo-HSCT.
CMV	See 'Cytomegalovirus'
Collection center	A medical center equipped to carry out collection of hematopoietic stem cells, either by peripheral blood stem cell apheresis or bone marrow harvest. Many will also undertake medical assessment of donors to establish fitness to donate.
Conditioning	A chemotherapy regimen with or without radiotherapy or immunotherapy designed to prepare the recipient bone marrow and immune system for receipt of donor HSC. Regimens may be myeloablative (full-intensity) or non-myeloablative (reduced-intensity).
Cord blood unit	A donation of blood, rich in HSC, derived from the umbilical cord and placenta of a newborn infant. Cord blood units are generally cryopreserved and may be used for allo-HSCT if found to be appropriately matched to a patient.
Cytomegalovirus (CMV)	A herpes virus prevalent in most populations, which generally causes a self-limiting illness in affected immunocompetent individuals. The virus persists asymptotically for life. However, in those with a compromised immune system, particularly following allo-HSCT, the virus may

	reactivate and cause severe morbidity and mortality.
Donor	Any individual, living or dead, providing solid organs, tissues or cells for the purposes of transplantation or transfusion. For the purposes of this thesis, a donor is an individual providing hematopoietic stem cells (either their own or cord blood HSC from a newborn infant) or lymphocytes for the allogeneic transplantation.
DFS	Disease free survival is defined as time from transplant to relapse, death or last follow-up
EBMT	The European Group for Blood and Marrow Transplantation
Ebstein-Barr Virus (EBV)	A herpes virus and one of the most common viruses in humans. The virus causes infectious mononucleosis. In most cases persists asymptomatic for life. In immunocompromised people is associated with post-transplant lymphoproliferative disease.
Engraftment	Evidence of hematopoiesis erasing from the donors HSC. Usually considered when absolute neutrophil count reaches $>0.5 \times 10^9/L$ for 2 consecutive days and platelets count $>20 \times 10^9/L$ without platelets transfusion.
Graft-versus-leukemia effect	A desired effect of allo-HSCT, describing “the immunological activity of the engrafted donor immune system against malignant recipient cells”.
G-CSF	Granulocyte colony-stimulating factor

Hematopoietic Stem cell (HSC)	A multipotent cell capable of self-replication and differentiation into cells of myeloid, erythroid or lymphoid lineage.
Haploidentical	A related donor who, whilst not being fully HLA-matched, is found to share one HLA haplotype in common with the potential recipient (and therefore is at least 5/10 matched).
Haplotype	In the context of HLA, the haplotype refers to a particular combination of HLA alleles encoded by one copy of chromosome 6. Thus any normal individual carrying two copies of chromosome 6 will have two HLA haplotypes. Haplotypes are usually inherited intact, so individuals will inherit one haplotype from each parent.
Histocompatible	The property of having a sufficiently similar set of HLA alleles to enable allogeneic transplantation.
HLA	Initially 'Human Locus-A, now usually an abbreviation of 'Human Leukocyte Antigen', HLA refers to the antigen system encoded by the Major Histocompatibility Complex that forms the cornerstone of the ability of the immune system to differentiate between 'self' and 'non-self'.
HSCT	Haematopoietic stem cell transplantation, which may be autologous (self-transplant), or allogeneic (donor, allo-HSCT).
HTA	See 'Human Tissue Authority'
Human Tissue Authority (HTA)	The UK legislative authority responsible for regulation pertaining to the handling of human cells, tissues and organs.
NIMA	Non-inherited maternal antigens
IPA	Inherited paternal antigens

NRM	Non-relapse mortality is defined as death without prior relapse
JACIE	Joint Accreditation Committee ISCT-EBMT. A collaborative global organization providing standards, inspection and accreditation for organizations involved in the collection, processing and transplantation of hematopoietic stem cells and related products.
KIR	Killer-immunoglobulin receptor
Major histocompatibility complex (MHC)	That region of chromosome 6 that encodes the chief determinants of histocompatibility and in particular the HLA molecules.
MiHA	Minor histocompatibility complex
MHC	See 'Major histocompatibility complex'
NK	Natural killer cells. Critical cells in innate immunity with cytotoxic potency to cancer and viral cells.
OS	Overall survival
Phenotype	In the context of HLA, the phenotype refers to a particular combination of HLA alleles in an individual. When matching at a 10/10 level, the phenotype describes the alleles present for each of two copies of HLA-A, -B, -C, -DRB1 and -DQB1.
Serology	In histocompatibility, the diagnostic investigation of antigens encoded by the MHC using antibody-based assays. However, serology also encompasses many other tests, such as

	identification of antibodies indicating exposure to infectious diseases.
SaBTO	Advisory committee on the safety of blood, tissue and organs
TC	See 'Transplant center'
TGS	Third-generation sequencing is a Pacific Biosciences SMRT (single-molecule real-time) DNA sequencing method.
TNC	Total nucleated cell count. It's a measure of the cell count reported after cord blood processing. TNC count is automated and measured by flow cytometer.
Transplant centre	A unit (usually within a hospital) capable of carrying out hematopoietic stem cell transplantation. For the purposes of this thesis, these centers carry out allogeneic HSCT.
Toxoplasma gondii	It's a parasite, which cause disease called Toxoplasmosis. It usually causes no symptoms in adults. In immunocompromised people disease might cause life threatening and debilitating organs damage.
Typing	In transplantation, typing is the process of establishing the HLA phenotype, and may include other non-HLA transplant determinants, such as KIR, NOD2, and CCR5.
UCBT	Unrelated cord blood transplant
WMDA	The World Marrow Donor Association. The WMDA was founded in order to provide a global forum for all matters relating to donors of hematopoietic stem cells.

Work-up

The process of preparing an unrelated adult donor for donation, generally involving logistic arrangements such as travel and accommodation, as well as a thorough medical examination, hematological and biochemical tests and infectious disease marker testing, in order to confirm the donor's fitness to donate.

Thesis overview

The primary aim of my thesis was to provide contemporary data on new factors that can influence the choice of unrelated umbilical cord blood units for transplantation. The results will help towards providing more targeted strategies for umbilical cord blood unit collection and selection, and I believe such strategies will improve overall transplant outcomes.

I started my research with a review of the literature, which is outlined in Chapter 1. This chapter explores the historical context of unrelated umbilical cord blood donation and gives an insight into factors that can influence umbilical cord blood units transplant outcomes.

The first study of this thesis, a prospective analysis of 2,411 UCBs stored at Anthony Nolan Cell Therapy Centre (AN CTC) between August 2012 and November 2013, explores the influence of delivery mode on UCB total nucleated cell dose (TNC) and volume.

In the second study of my thesis I focused on an evaluation of the extent of HLA mismatch at high resolution (for HLA-A, -B, -C and -DRB1 genes) between 208 UCB-recipient pairs for whom work-up for UCBT was requested via Anthony Nolan between 1st January 2013 and 31st December 2015 from 12 UK Transplant Centres. My study revealed that, some units showed substantial HLA disparity with the recipient when typed to high resolution for HLA-A, -B, -C

and –DRB1 genes, with units as low as 3/8 HLA match being used for transplant. The transplant centres should be aware of the possibility of choosing “sub-optimal “cord blood units for transplantation when applying HLA matching at the “classical level” (i.e. low/intermediate level for HLA Class I genes (-A and-B) and high resolution for HLA Class II genes (mainly-DRB1)). This is particularly important when selecting the cord blood unit for a single unit UCBT.

In the third study of my thesis, I designed a retrospective study which explored a possible correlation between the cell counts of 14,408 UCBs (4,654 UCBs from AN CTC and 9,754 UCBs from Barcelona’s Cord Blood Bank (CBB)) and their donors history of exposure or non-exposure to infections (in particular CMV, Epstein-Barr Virus (EBV) and *Toxoplasma gondii*). The study included cord blood units stored by both banks between 1st October 2008 and 30th September 2014.

I subsequently designed the prospective study in collaboration with Eurocord, as described in Chapters 5 and 6. As part of the preparation for my prospective study and to ensure the adequate capture of my outcome data, I spent one week in Eurocord’s main office in Paris, working closely with staff members.

This study is the core of my thesis and assesses the influence of maternal CMV serological status and previous pregnancies on unrelated cord blood units transplant (UCBT) outcomes for recipients with acute leukaemia between 1st January 2000 and 31st December 2012. Chapter 5 explains the methodology behind the study described in Chapters 6. Chapter 6 provides an analysis of the

impact of maternal CMV serological status and provides an analysis of the influence of maternal previous pregnancy on single-unit UCBT on the single-unit UCBT outcomes for 1177 paediatric and adult patients with acute leukaemia.

Chapter 7 summarises the findings of this thesis and describes some of the challenges encountered, and future plans to expand on this work.

Chapter 1. Introduction and literature review

1.1. History and Context

1.1.1. *Umbilical cord blood (UCB) as a source of stem cell procurement*

Barnes and *Loutit* proposed the idea of the potential curative effect of bone marrow transplantation for malignant diseases back in the late 1950^s. They had observed an “anti-leukaemic effect of transplanted spleen cells in experimental murine models” (On & Block, 1956). In 1959, the first human bone marrow transplant proved the possibility of haematological reconstitution in lethally irradiated patients with acute leukaemia. Early on, results were not promising, mainly due to the high death rate of graft-versus-host disease (GvHD), graft failure or disease relapse (Mathe, Amiel, Schwarzenberg, Cattani, & Schneider, 1963), (Mathé, Amiel, Schwarzenberg, Catran, & Schneider, 1965). The major breakthrough in the success of human bone marrow transplantation came as a result of the pioneering work of both *Dausset* and *Van Rood*, who discovered the HLA system. This opened up a new era in the understanding of haematopoietic stem cell (HSC) immunogenicity.

For a long time, only two major HSC sources were used for transplantation – bone marrow (BM) and granulocyte colony stimulating factor (G-CSF) mobilised peripheral blood stem cells (PBSC). In 1982, *Hal Broxmeyer* suggested using umbilical cord blood as a source of allo-HSC graft. *E. Gluckman's* group brought

it to clinical practise in 1988 by successfully infusing a sibling's UCB into a child with *Fanconi* anaemia (Ballen, Gluckman, & Broxmeyer, 2013). This event marked the beginning of a new field in allo-HSCT. Since then, there has been a significant increase in understanding of UCB immunology, collection, processing and cryopreservation, thawing and shipping around the world (Bradley & Cairo, 2005).

Large numbers of transplant centres (TCs) have reported successful related and unrelated umbilical cord blood unit transplants (UCBTs) with transplant outcomes comparable to those using other HSC sources. *Grewal et al.*, performed a matched-pair analysis comparing the outcomes of (predominantly paediatric) recipients having both malignant and non-malignant disorders and 0 to 3 HLA-mismatched UCB with HLA-A, -B and -DRB1 matched BM) (*Grewal et al.*, 2003). The study showed comparable results in acute and chronic GvHD rates and overall survival OS. A similar study was performed in Europe on 541 children with acute leukaemia. Patients received either unrelated donor BM or T-cell depleted UCBT. The study showed comparable results for 2-year OS rate (49% and 41% respectively) and event-free survival rate (43% and 37%) in the unrelated donor BM and UCB recipients (*Rocha et al.*, 2001). Results of other studies comparing umbilical cord blood to other graft sources in adults and children are summarised in Table 1-1.

Table 1-1: Results of comparative studies on BMT, PBSCT and UCBT in adults and children

Study Population (N)	Graft Source (N)	Conditioning	Results	Reference
Adults with haematological malignancies (113)	UD (45) UCB (68)	MAC	-Delayed neutrophil and platelets engraftment -Decreased TRM and DFS	(Takahashi et al., 2004)
Adult with haematological malignancies (1280)	UD (1132) UCB (9148)	MAC	-Delayed neutrophil engraftment (<i>versus</i> BM) -Increased NRM -Comparable OS and DFS (<i>vs</i> BM)	(M. Eapen et al., 2010)
Children with HS (258)	MSD (37) UD (105) UCB (116)	MAC	-Higher enzyme levels and full donor chimerism (<i>versus</i> MSD) -Comparable OS (with MSD) n	(Boelens et al., 2010)

Children with malignant and non-malignant conditions (1180)	MSD (469) UCB (711)	MAC (55%) RIC (45%)	Decreased cGvHD	(Brunstein et al., 2010)
Adults with haematological malignancies (285)	dUCB (64) UD (221)	RIC	-Decreased cGvHD -Higher NRM -Comparable OS and DFS	(Chen et al., 2012)
Adults with haematological malignancies (556)	UCB (112) UD (334)	MAC	-Pre-transplant MRD does not have influence on RR after UCBT -Lower RR	<i>Milano et al, ASH 2014</i>
Infants with SCID (240)	UCB (43) UD (197)	RIC(35) MAC(46) None (120) IS (39)	Decreased OS	(Pai et al., 2014)
Adults with haematological	dUCB (128)	MAC	Lower RR	(Lazaryan et al., 2015)

malignancies (536)	MSD (204) UD (204)			
<p>BMT: bone marrow transplantation; PB SCT: peripheral blood stem cell transplantation; UCBT; umbilical cord blood transplantation; MAC: 32yeloablative conditioning regimen; RIC: reduced intensity conditioning regimen; MSD: matched sibling donor; UCB: umbilical cord blood; dUCB: double umbilical cord blood; aGvHD: acute graft-versus-host disease; cGvHD: chronic graft-versus-host disease; UD: unrelated donor; NRM: non-relapse mortality; OS: overall survival; DFS: disease- free survival; SCID: severe combined immunodeficiency; IS: immunosuppression; HS: Hurler syndrome; RR: relapse rate</p>				

Compared to BM and PBSC, UCB offers several advantages over adult HSC sources. These include: no risk to the donor, no donor attrition, immediate availability of fully tested and HLA-typed UCB in a frozen state when HSCT is required, lower risk of transmitting infections and the possibility of cryopreserved storage for more than 20 years with efficient recovery of HSC (Gluckman & Rocha, 2006 and),(Stanevsky, Goldstein, & Nagler, 2009) .

Also, UCB banks have a greater proportion of rare HLA phenotypes, which is extremely relevant for ethnic minority patients in need of HSCT (Boo, Ballen, & Maiers, 2011). Last but not the least; high quality UCB decreases the probability of chronic GvHD in comparison with other graft sources (Boelens et al., 2010), (Y. Cohen & Nagler, 2004).

“Development of moderate to severe acute or chronic GvHD is associated with diminished quality of life, as well as decreased overall survival” (Nash et al., 1992). “Grades II to IV acute GvHD is reported in 43% to 70% of matched adult

unrelated donor (MUD) BM transplants, and in 63% to 95% of single HLA-antigen mismatched adult unrelated donor transplants. Chronic GvHD affects more than 55% of MUD transplant recipients and as many as 80% of those receiving single HLA antigen mismatched unrelated donor grafts” (Martin et al., 2006). UCBT studies have reported incidences of 33% to 44% grades II to IV and 11% to 22% grades III to IV acute GvHD respectively, and a 0% to 25% incidence of chronic GvHD (Rubinstein et al., 1998), (Wagner et al., 2002). Table 1-2 lists main advantages and limitations of umbilical cord blood unit in comparison with other stem cell sources.

Table 1-2 : Advantages and limitations of umbilical cord blood unit in comparison with bone marrow or peripheral blood stem cells

Advantages	Limitations
Prompt availability	Limited cell dose per recipient's weight
No risk to the donor	Delay in neutrophil and platelets engraftment
No donor attrition	No chance for the second donation/donor lymphocyte infusion
No risk of donor attrition	Lack of transfer of T and B memory cells
Reduced risk of chronic graft-versus-host disease	Theoretically increased risk of transferring hereditary disorders
More HLA-mismatch tolerance is observed	Slower immune reconstitution if serotherapy given
Low risk of viral contamination	
Better representation of ethnic minorities	
Long-term cryopreservation	

Easy to schedule or re-schedule transplantation

Due to the development of safer protocols for T-cell replete haploidentical transplantation, the use of family mismatched donors has increased over the recent years. This approach seems to be very attractive to the clinicians, because it is relatively easy and quick to schedule, almost every patient has a suitable donor and the financial burden to the transplant centre is moderate. Recently, the EBMT and Eurocord group compared outcomes of single or double UCBT with T-cell replete haploidentical transplantation in patients receiving either 34yeloablative (MAC) or reduced intensity (RIC) regimen for patients with haematological malignancies (A. Ruggeri et al., 2015). No statistically significant difference was found between the two graft sources for leukaemia-free survival, overall survival, relapse incidence or transplant related mortality. However, UCBT was associated with delayed engraftment but a lower incidence of chronic GvHD in the multivariate analysis.

One of the major limitations of UCBT which is mentioned above is “delayed engraftment and slower immune reconstitution owing to the qualitative and quantitative characteristics of UCB, such as naïvity of cord blood T- cells and a fixed cell count” (L. Tucunduva et al., 2014), (Danby & Rocha, 2014). The influence of UCB quality parameters on UCBT outcome will be outlined in the following sections of this chapter.

1.2. UCB Banking

When bone marrow transplantation became an effective and feasible curative approach for many patients, the need for unrelated bone marrow donor registries was identified too, because not everyone could find a HLA-matched sibling or other family donor. The first unrelated bone marrow donor registry was established in London in 1974, by *Shirley Nolan*, whose son Anthony unfortunately passed away, without the chance of being cured, from immunodeficiency. Since that time, the number of registries has continued to grow and currently over 25 million unrelated donors are registered worldwide (“WMDA - Home,”).

The first cord blood banks (CBB) for unrelated and related units were established in 1993 in New York, Milan and Dusseldorf (Bradley & Cairo, 2005). The first CBB in the UK was established in 1996 by National Health Services Blood and Transplant (NHSBT). Although the management of available CBB varies between countries, certain rules for the quality of the system and product apply. Organisations such as The American Association of Blood Banks, European Blood and Marrow Transplantation Society, Eurocord, NETCORD and FACT are in place to ensure that the quality and standards for minimal volumes collected, sterile processing, red blood cell depletion, volume reduction, measurement of CD34+ count and other cell subsets and cell recovery after processing are established (*EBMT-ESH handbook 6th Edition, 2012*), (Bradley & Cairo, 2005). According to the Bone Marrow Donors Worldwide (BMDW) database, 53 cord blood registries from 36 countries with

684,896 CBUs are registered (as of March 2016) (“BMDW: Home,”) (see Figure 1-1).

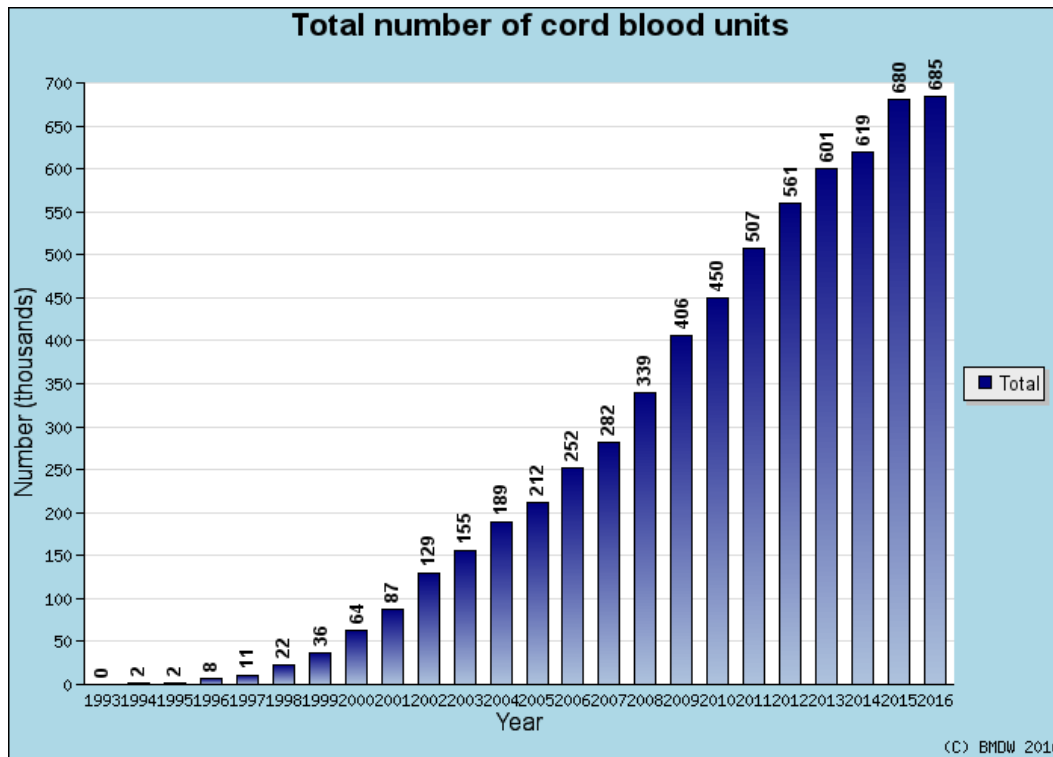


Figure 1-1: The number of banked Cord Blood Units Worldwide (“WMDA-Home”)

“Establishment of CBBs has resulted in reduced search times for unrelated cord blood donors compared to adult donor HSC sources” (Mayani, 2011).

In the modern era, cost management plays an increasing role in determining UCB selection and use. Therefore, it is of paramount importance to understand when and how UCBT can be utilised in the most beneficial way for both the recipient and TC. Characteristics of UCB that influence the banking and unit selection for HSCT will be outlined in the following sections of this chapter.

1.3. Factors known to influence Umbilical Cord Blood Transplant Outcomes

1.3.1. Introduction

The current selection strategies for the optimal allo-HSCT graft include considerations such as donor age, preferred method of donation, gender, infectious markers (in particular CMV) and HLA-type. Recipient considerations include: disease type (malignant *versus* non-malignant), age, concomitant morbidities and characteristics and type of the conditioning regimen (reduced intensity *versus* 37yeloablative) used in preparation for the transplant. Of primary importance is sufficient HLA matching between donor and recipient to ensure engraftment and acceptable rates of GvHD. Another important factor is the donor and recipient CMV status, which plays a crucial role in HSCT outcome (Green et al., 2013a), (Schmidt-Hieber, 2013). Other factors that may be considered are recipient-graft gender and ABO mismatch. Sometimes, length of time to transplant can be crucial for successful transplant outcome. The rapid donor identification and imminent availability of UCB grafts gives patients with high-risk diseases a better chance of success.

As mentioned previously, “UCBT can be associated with delayed engraftment, poor immune reconstitution and higher rates of acquired infections compared to other HSC sources” (Danby & Rocha, 2014), (Gluckman et al., 2004). Three major UCB related factors that have been associated with transplant outcome are: total nucleated cell count (TNC), CD34+ dose per recipient’s weight and

level of HLA-matching. These factors are interconnected and will be described below.

1.3.2. *TNC and UCB Selection*

“While UCB contains a higher concentration of HSC than adult peripheral blood, each unit contains a one or two log lower TNC dose in relation to recipients size compared to BM and PBSC” (Danby & Rocha, 2014). On average, a UCB unit contains $0.5-3 \times 10^9$ TNC. The time taken to achieve haematopoietic recovery post-transplant, identified by recovery of donor-derived neutrophil and platelet count, directly depends on TNC infused. Median time to neutrophil engraftment (count $>0.5 \times 10^9/L$ for 2 consecutive days) post single-unit UCBT is as long as 28 days and 90 days for platelets (count $>20 \times 10^9/L$ without transfusion) (Rubinstein et al., 1998). A Eurocord study showed that a TNC dose higher than $3.7 \times 10^7/kg$ of recipient’s weight is associated with shorter time to neutrophil recovery: 25 versus 35 days (Gluckman et al., 2004). In addition, several studies have shown that the number of TNC infused is associated with lower transplant related mortality (TRM) and better OS rates (Gluckman et al., 2004) (Rocha & Gluckman, 2009). There is an overall consensus that a minimal TNC dose of $2.5 \times 10^7/kg$ of recipient’s body weight is an accepted minimum standard in clinical practise for optimal engraftment (V Bachanova et al., 2014).

Therefore the time to haematopoietic recovery might be prolonged (Gluckman et al., 2004). This exposes the recipient to an increased risk of acquired infections. The optimum timing of measurement and actual number of TNC

required still remains under discussion. Most published studies report the impact of pre-freeze TNC dose on transplant outcome. However, one should be aware that after processing and thawing of UCB, up to 20% of TNC will be lost (Hough et al., 2015). Therefore, knowing the actual dose at infusion (post-thaw) becomes more important. The fixed cell count in UCB represents the major limiting factor, particularly for adult recipients with greater disparity of weight/size between UCB and patient. Therefore, for many years the advantages of UCBTs were only being appreciated in the paediatric population, where outcome was comparable to that of transplantation from adult unrelated donors (M E Horwitz, 2014).

Relatively lower cell dose per recipient's weight was the main obstacle for performing UCBT in adults. For most adult recipients, a single-unit UCBT is almost always insufficient to provide enough cell dose. However, recent data from the Eurocord registry showed that, after 2004, the number of adults transplanted using UCB per year has increased (Figure 1-2) to reach a peak in 2011, with a slight drop in numbers after that time. This increase is might to be related to the possibility of use of double-unit cord blood transplantation (Majhail, Brunstein, & Wagner, 2006), demonstration that reduced intensity conditioning regimens are safe in the context of cord blood transplantation (Barker et al., 2003), advances in UCBT unit selection, growing experience of transplant centres and improvement in supportive care (Annalisa Ruggeri, 2016).

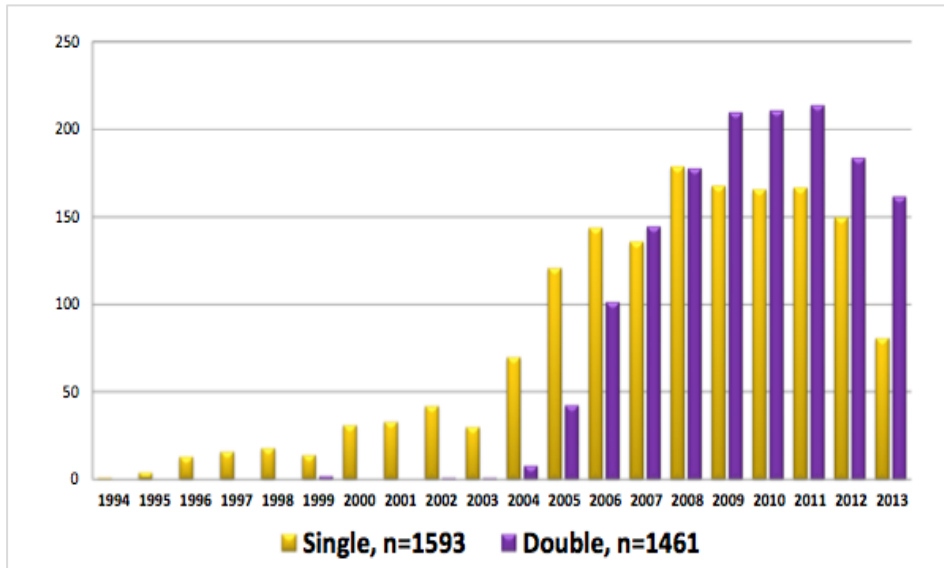


Figure 1-2: Number of UCBT in adults per year reported to Eurocord (Annalisa Ruggeri, 2016)

Table 1-3 provides an overview of the studies that have evaluated the impact of TNC dose of the single-unit infusion to UCBT outcomes in both adult and paediatric recipients.

Table 1-3 : Overview of the studies that evaluated influence of TNC dose on UCBT outcomes after single UCB infusion

Study population (N)	Objectives	Outcomes
Eurocord study on both adults and paediatric recipients with malignant and non-malignant disorders (925) (Gluckman & Rocha, 2006)	To evaluate influence of cell dose and HLA matching on UCBT outcomes	Median infused TNC dose of $3.7 \times 10^7/\text{kg}$ associated with better platelets and neutrophil engraftment and overall survival

<p>Paediatric recipients with mainly non-malignant disorders (562) (Rubinstein et al., 1998)</p>	<p>To evaluate influence of cell dose on UCBT outcomes</p>	<p>-Speed of neutrophil engraftment positively correlated with the leukocyte cell dose in the graft -Pre-freeze cell dose < $2.4 \times 10^7/\text{kg}$ was associated with higher mortality rate</p>
<p>Adult recipients with haematological malignancies (68) (Laughlin et al., 2001)</p>	<p>To evaluate the cell dose on UCBT outcomes</p>	<p>Median pre-freeze TNC dose of $2.4 \times 10^7/\text{kg}$ associated with better neutrophil engraftment</p>
<p>Adult recipients with haematological malignancies after MAC (171) (Arcese et al., 2006)</p>	<p>To evaluate the transplant outcomes</p>	<p>A TNC dose > $2.7 \times 10^7/\text{kg}$ was associated with faster neutrophil engraftment</p>
<p>Adult recipients with ALL after MAC (27) (Ooi et al., 2009)</p>	<p>To evaluate transplant outcomes</p>	<p>TNC dose was not associated with faster neutrophil engraftment</p>
<p>Eurocord study on adults 925 recipients with malignant (925) and non-malignant (279) haematological disorders (Danby & Rocha, 2014)</p>	<p>To evaluate transplant outcomes</p>	<p>-Pre-freeze TNC dose of minimum $3 \times 10^7/\text{kg}$ associated with better transplant outcomes for malignant disorders</p>

		<p>- Pre-freeze TNC dose of minimum $4.9 \times 10^7/\text{kg}$ associated with better transplant outcomes for non-malignant disorders.</p>
<p>ALL: acute myeloid leukaemia; MAC: myeloablative conditioning; RIC: reduced intensity conditioning; TNC: total nucleated cell dose</p>		

In order to overcome cell dose limitations for adult patients, double-unit infusions were suggested. Studies on HSCT with double-unit UCB showed a cumulative incidence of neutrophil recovery of 91% at a median of 21 days (Wagner, 2009). Both units contributed to early engraftment but only one unit predominated later (Barker et al., 2005). Data relating to quality characteristics of the dominant unit are sparse, regarding better CD3+ cell dose (Barker et al., 2005) or higher CD34+ cell dose (Purtill et al., 2014). In addition to the potential for improved engraftment, some studies “demonstrated a decrease in relapse rate when two UCB units were used, possibly due to a superior graft-versus-leukaemia (GvL) effect” (Ballen & Lazarus, 2016), (Verneris et al., 2009). However, a recent report has shown similar outcomes in terms of disease-free survival, neutrophil engraftment, transplant related mortality and incidence of relapse in 224 children and adolescents, recipients of either single- (n=113) or double-unit (n=111) UCBT for haematological malignancies, providing that single UCB contains sufficient TNC dose (Wagner et al., 2014). Similar results were observed in another study on 151 paediatric patients with acute

leukaemia, who received single – (n=74) or double-unit (n=77) UCBT (Michel et al., 2016).

Other approaches to overcome the limitations of cell dose include “*in vivo* and *ex-vivo* expansion of UCBs using growth cytokines, improving UCB homing, addition of third-party mesenchymal cells, intrabone injection of HSCs and co-infusion with haploidentical T-cell depleted graft” (Delaney, Ratajczak, & Laughlin, 2011) (Mitchell E. Horwitz & Frassoni, 2015). Table 1-4 provides an overview of studies that implement *ex vivo* expansion of the umbilical cord blood unit and stem cell manipulation before transplantation.

Table 1-4 : Overview of the studies exploring UCB expansion

Study	Compound	Modality	Clinical trial
Delaney (Delaney et al., 2011)	Notch ligand	<i>Ex vivo</i> expansion	Yes
Horwitz (Mitchell E. Horwitz & Frassoni, 2015)	Nicotinamide (NiCord)	<i>Ex vivo</i> expansion	Yes
Peled (Peled et al., 2004)	Copper chelation	<i>Ex vivo</i> expansion	Yes
Cutler (Cutler et al., 2013)	Prostaglandin E2	Enhancing homing	Yes
Robinson (Robinson et al., 2011)	Mesenchymal cell co-culture	<i>Ex vivo</i> expansion	Yes

Brunstein (Brunstein et al., 2013)	Complement fragment 3a co- culture	<i>Enhancing homing</i>	Requires more data
Robinson (Robinson et al., 2012)	Fucosylation	Enhancing homing	Yes
Wagner (Wagner et al., 2016)	StemRegenin1	Stem cell expansion	Yes
Shpall (Shpall, 1999)	Cytokine (SCF, TPO, Flt-3L, G- CSF, MGDF)	Stem cell expansion	Yes
Farag (Farag et al., 2013)	Inhibition of CD26/DPP-4	Enhancing homing	Yes

“Clinical data on these techniques are already available and short-term safety has also been demonstrated” (Mitchell E. Horwitz & Frassoni, 2015). However, long-term follow up is required for confirmation of these results. “No randomised studies have been completed to date to demonstrate improved survival rates with expanded *versus* unexpanded cells” (Ballen & Lazarus, 2016). Nevertheless, these methods look like an extremely attractive approach for clinicians. However, because of the logistical and financial complexity associated with production of a patient-specific expanded UCB graft, this approach is not widely implemented in routine clinical practice (Mitchell E. Horwitz & Frassoni, 2015).

As selection of the optimal and appropriate UCB graft is a more complex process than selecting the most suitable BM or PBSC donor, the British Society of Bone Marrow Transplantation (BSBMT) Cord Blood working Group (CBWG) published UK consensus recommendations for selection of individual UCB grafts. According to latest guidelines published in 2015, the minimum recommended TNC count at freezing should be $3.0 \times 10^7/\text{kg}$ of recipient's weight for malignant disorders and $3.5 \times 10^7/\text{kg}$ for non-malignant disorders in the case of single-unit UCBT. When a single unit contains an insufficient cell count, double-unit infusion is recommended. The recommended total TNC count at freezing should be $> 3.5 \times 10^7/\text{kg}$, with a minimum TNC count of each unit being $1.5 \times 10^7/\text{kg}$ (Hough et al., 2015). A combination of cell dose and HLA-matching, as well as recipient's condition (malignant *versus* non-malignant disease) should also be taken into consideration.

1.3.3. UCB selection and CD34+ cell count

The CD34+ cell count represents and reflects the actual content of immature haematopoietic progenitors in the graft. "The loss of CD34+ cells after thawing is less important, therefore, the number of CD34+ cells at freezing might be considered when selecting a UCB graft for transplant" (Rocha & Gluckman, 2009). Some studies have revealed a "less mature phenotype of UCB CD34+ cells compared to adult marrow and peripheral blood grafts but with higher proliferative potential" (Danby & Rocha, 2014). In a series of 102 adult allo-UCBT recipients, in 2002, *Wagner et al* observed a "significantly inferior speed and probability of engraftment with a CD34+ cell dose lower than $1.7 \times 10^5/\text{kg}$.

Moreover, the group showed a decreased OS rate in patients who received a CD34+ cell dose which was less than $1.7 \times 10^6/\text{kg}$, compared to patients who had received a CD34+ cell dose greater than $1.7 \times 10^6/\text{kg}$ (29% *versus* 68% respectively)" (Wagner et al., 2002). In 2013, the Anthony Nolan (AN) Cell Therapy Centre (CTC) performed a retrospective study within WMDA members to evaluate the parameters considered to be most important when selecting UCB grafts. Both providers (CBBs) and selectors (TCs and registries) were sent a survey. Interestingly, out of 50 responders who submitted a completed survey, UCB graft selectors did not indicate that the CD34+ pre-freeze or post-thaw cell dose was a top requirement (*personal communication with Dr S G Gomez, former head of AN CTC*). Unfortunately, the absence of a standardised counting method between centres precludes CD34+ cell count from being used for comparative clinical studies.

According to the BSBMT CBWG guidelines, the minimum recommended CD34+ cell dose for malignant disorders should be $1.1\text{-}1.7 \times 10^5/\text{kg}$ of recipient's weight pre-freezing and around $1.0\text{-}1.2 \times 10^5/\text{kg}$ after thawing. In the case of non-malignant disorders, the recommended CD34+ cell dose should be $>1.7 \times 10^5/\text{kg}$ of recipient's weight at freezing or after thawing. In the case of double-unit selection, the recommended total dose at freezing or after thawing should be $>1.8 \times 10^5/\text{kg}$ (Hough et al., 2015).

1.3.4. UCB graft selection and non-inherited maternal antigens (NIMA) and inherited paternal antigens (IPA)

Recent studies have indicated that alloreactivity in transplantation involves not only direct donor-recipient reactivity but also maternal and paternal reactivity.

During pregnancy, bi-directional transplacental cell trafficking results in UCB containing cells derived from both the foetus and the mother. Maternal cells are sensitised against HLA antigens that the fetus has inherited from the father (IPA) and cord blood cells are sensitised to those not inherited from mother (NIMA) (Van Rood et al., 2012). Because of the existence of fetomaternal tolerance, non-inherited paternal antigens are more immunogenic than NIMA (Hirayama, Azuma, & Komada, 2012). Anti-IPA immunity in the mother can persist for many decades after delivery and passes to other siblings during subsequent pregnancies (Dierselhuis et al., 2012). Please see Figure 1-3 for schematic explanation of the NIMA and IPA immunogenicity.

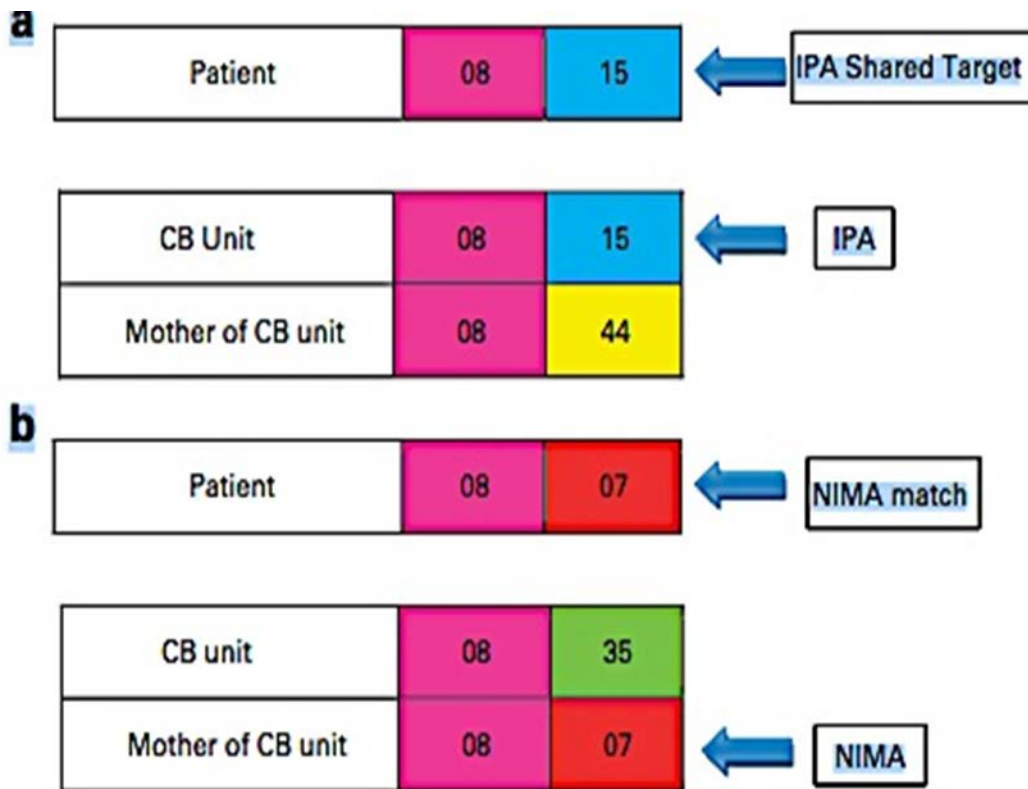


Figure 1-3: IPA and NIMA in UCB (Milano, Lee Nelson, & Delaney, 2013)

(a) “HLA typing is used as an example to illustrate the IPA in cord blood transplantation (CBT). Anti-relapse benefit is predicted in the example illustrated because the mother’s T cells would be sensitized to the HLA-B antigen encoded by HLA-B*15 (IPA) and the cord blood (CB) recipient has HLA-B*15 (shared IPA target). Patient and donor CB unit match for B antigens encoded by HLA-B*08 and HLA-B*15. Based on the HLA typing of the CB and its mother, HLA-B*15 is the IPA in the CB. In this case, the patient also has HLA-B*15, so IPA-sensitized maternal cells have a target.”

(b) “Decreased transplant related mortality was reported for patients receiving one or two antigen-mismatched CBT when the patient had the same HLA antigen as the CB’s NIMA. Patient and donor CB unit are mismatched at the HLA-B locus: patient has HLA-B*07 while CB has HLA-B*35. However, the patient’s mismatched HLA-B*07 matches the CB’s NIMA at the same locus” (Milano, Lee Nelson, & Delaney, 2013).

Several studies have been performed to investigate the influence of NIMA and IPA on transplantation and outcome. There is a hypothesis suggesting that HLA-mismatches which are matched to the donor’s NIMA might be permissible

after UCBT (van Rood et al., 2009). One study showed that single-unit UCBT recipients with acute myeloid malignancies, who were NIMA matched to the UCB, showed lower relapse rate and better OS (van Rood et al., 2009). To date, the effect of NIMA matching in double-unit UCBT has not been investigated. In clinical practise, when no fully matched UCB graft is available, an HLA-mismatched graft, which is NIMA-matched, is preferred over an HLA-mismatched graft which is NIMA-mismatched. If it is possible to implement NIMA matching in UCB graft selection, it might further enlarge the pool of suitable unrelated UCB grafts in the future. Currently, not all CBB have maternal HLA typing available.

The same mechanism is observed with paternal antigens. Microchimeric maternal cells that are sensitised toward fetal IPA during pregnancy can be co-infused with UCB. Therefore, maternal immunity towards IPA might influence UCBT outcome. "Patients with acute myeloid or lymphoid malignancies who share one or more antigens with their UCB graft's IPAs had a significant decrease in leukemic relapse post UCBT (HR=0.38, $p<0.001$) compared with those that did not" (Rood et al., 2012). Like maternal HLA typing, paternal HLA typing is most likely not performed at all. Further clinical data is needed in order to implement it in routine practice.

1.3.5. UCB selection and Killer-immunoglobulin-like receptor (KIR) ligands

“Natural killer (NK) cells are critical to the innate immune system because they mediate cytotoxic lysis to cancer and virally infected cells” (Yoo et al., 2007) .

In the adult donor HSCT setting, NK cells play an important role in mediating the GvL effect (L. Ruggeri et al., 2007). Among NK cell receptors, inhibitory killer cell immunoglobulin-like receptors (iKIR) are of paramount importance because of their ability to recognise ubiquitously expressed host MHC class I ligands, and their regulatory role due to NK cell education or licensing (Kim et al., 2005). When donor NK cells lack inhibitory KIRs on their cellular surface which interact with recipient HLA ligands (in particular HLA-C molecule in the C1 group), the donor is considered KIR ligand mismatched (Sekine et al., 2016). In a study of 218 patients with acute leukaemia, recipients who had received single-unit UCBT, donor KIR ligand incompatibility in the GvHD direction was associated with decreased relapse incidence (HR=0.53, p=0.05) and improved leukaemia-free survival (HR=2.0, p=0.0016). Effect was more evident for AML transplant recipients (Willemze et al., 2009). However, a recent study of 461 patients with acute myeloid leukaemia treated with a MAC single-unit UCBT showed no difference in relapse incidence, non-relapse mortality and overall survival based on KIR ligand match/mismatch status. However in recipients of grafts with three or more HLA mismatches, KIR ligand mismatched grafts provided lower non-relapse mortality (HR=2.26, p=0.008) (Rocha et al., 2016). *Sekine et al., in 2016* showed on a group of 110 recipients of single-unit UCBT

for haematological malignancies that optimal CBU-recipient KIR-HLA genotype was an independent predictor factor of transplant outcome. Patients homozygous for HLA-C2 group had a higher relapse rate and inferior overall survival in comparison to patients with HLA-C1/C1 or HLA-C1/C2 groups (68.8%, 26% and 15% respectively, $p=0.001$) (Sekine et al., 2016). The authors suggest that effect is explained by NK cells licensing and alloreactivity. On the basis of these data, KIR-ligand matching might become an important factor in current UCB graft selection criteria.

1.4. Other factors

1.4.1. Functional assays of UCB quality

Graft potency is the ability of HSC to engraft. It is usually assessed by cell viability on flow cytometry in both TNC and CD34+ compartments. Some researchers reported an association between good CD34+ and TNC viability, and improved transplant outcome (Barker et al., 2009). Unfortunately, as with CD34+ cell count assessment, there are no standardised methodologies to measure this.

Another surrogate marker of HSC actual clonogenic potential, which can be used, is enumeration of granulocyte–macrophage colony-forming units (CFU-GM) in UCB. Unfortunately, different methods of cell isolation and growth exist between laboratories. Currently there are controversial reports on the clinical significance of CFU-GM count. A group from the US performed analysis on the

predictive value of CFU-GM across four US cord blood banks. They did not find any predictive value to the assay which would correlate with neutrophil engraftment (van Besien, 2014). Other studies showed an inverse correlation between the dose of GM-CFC and time to engraftment (Migliaccio et al., 2000). Given the fact that this correlation was not always reproducible, this marker is very difficult to apply in routine practise.

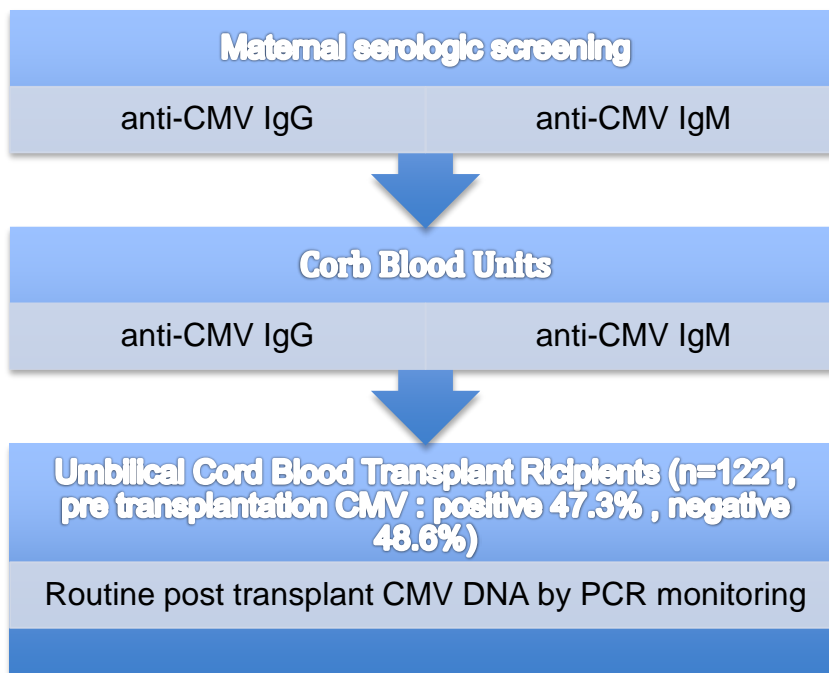
Recently, based on the correlation between CFU-GM and CD34+ cells, the Barcelona group has proposed the use of the clonogenic efficiency (CLONE, i.e. the ratio between post-thaw CFU-GM counts and pre-freezing CD34+ cell numbers) as a new measure of HSC potency (Castillo et al., 2015). The group has studied the outcomes of 110 adult patients with haematological malignancies who were recipients of a single-unit UCBT. CLONE of $\geq 20\%$ predicted a faster neutrophil ($p=0.005$) and platelet ($p=0.02$) engraftment and contribution to a decrease in the non-relapse mortality ($p=0.02$). In addition, they found that the infusion of $>2.0 \times 10^7/\text{kg}$ of recipient's weight post-thaw of viable CD45+ cells "was significantly associated with faster neutrophil ($p=0.01$) and platelet ($p=0.01$) engraftment, higher disease-free survival ($p=0.01$) and OS ($p=0.02$)" (Castillo et al., 2015).

1.4.2. *Cytomegalovirus*

CMV is the most common blood product –transmitted virus. Prior to arrival of effective antiviral therapy in early 1990, cytomegalovirus (CMV) disease

was the leading infectious cause of death among CMV-seropositive recipients of HSCT (Hannachi et al., 2011). In the context of adult unrelated HSCT, the pre-transplantation CMV status of the donor and recipient is an important risk factor for post-transplantation outcome (Ljungman, Hakki, & Boeckh, 2010). Several cohort studies showed that CMV seropositive transplant recipients and seronegative recipients of CMV positive grafts appeared to have higher transplant related mortality when compared with seronegative recipients with a seronegative donor (Boeckh & Nichols, 2004). “Umbilical cord blood is presumed to have a low risk of transmitting CMV because of the low rate of congenital CMV infection (reported to 0.2% to 2.5% depending on socio-economic status and geographical location)” (Griffiths, Baraniak, & Reeves, 2015). Options for CMV screening for UCB include serologic testing of the donor infant’s mothers or serologic testing of the UCB. “Interpretation of data obtained by serologic methods alone is complicated in cord blood donors because of active transport of maternal IgG antibody across the placenta. Thus, CMV antibody positivity in cord bloods generally reflects the mother’s lifetime exposure to CMV” (Albano et al., 2006). In 2006, *Albano et al* had assessed the incidence of post-transplantation CMV infection in recipients of UCB donated to the New York Blood Centre National Cord Blood Program (Albano et al., 2006). Figure 1-4 displays the diagram of the study.

Figure 1-4 : Diagram of the study on the incidence of CMV infection post UCBT



As a result of the study, it was determined that there was no association between the UCB donor's maternal CMV antibody status and incidence of post-transplantation CMV infection. In line with previous studies, "the incidence of post-transplantation CMV infection was associated with recipient's pre-transplantation CMV serology, age, HLA mismatch and ethnicity. This was the first study that evaluated whether there is an association between UCB donor serology for CMV antibody and post-transplantation CMV infection in the recipient. It showed for the first time that unlike studies on adult donors' recipients, post-transplantation CMV infection in UCB recipients had no association with donor serology (i.e., the donor mother's CMV antibody status)" (Albano et al., 2006).

An effect of CMV infection on the relapse rate post transplantation for malignant disorders was described in adult recipients of unrelated adult donors. In adult recipients a lower incidence of relapse of acute myeloid leukaemia was associated with CMV reactivation (Elmaagacli et al., 2011) (Green et al., 2013b)(Ito et al., 2013). This effect was thought to be attributed to the NK cells alloreactivity and potential Graft Versus Leukaemia effect. Conversely, a very recent large retrospective study from the CIBMTR showed that CMV reactivation had no preventative effect on haematologic disease relapse and was associated with a higher non-relapse mortality (Teira et al., 2016). To date, only one study has been published on the incidence of leukaemia relapse with post- transplant CMV infection when UCB was graft source in paediatric recipients (Jeljeli, Guérin-El Khourouj, Porcher, Fahd, Leveillé, Yakouben, Ouachée-Chardin, Legoff, et al., 2014). In contrast to published studies on adult acute myeloid leukaemia, CMV reactivation was associated with increased relapse rate in this paediatric series. Of note, UCB was the stem cell source only in 16% of the patients. Therefore the role of CMV infection post UCBT in relapse incidence rate is to be yet explored.

1.4.3. UCB selection, donor-specific HLA allo-antibodies and ABO mismatch

Pre-formed donor-specific HLA allo-antibodies play a role in transplant outcome, “owing to the higher risk of graft failure and increased GvHD risk” (Spellman et al., 2010). Antibodies against the UCB HLA have been reported to have a negative impact on neutrophil and platelet engraftment in a single-

unit UCBT setting (Takanashi et al., 2010). With regard to double-unit UCBTs, study results are inconclusive. “Although not replicated in all centres, many institutions performing UCBT will exclude an UCB unit for which the patient has pre-formed HLA-specific antibodies” (Ballen & Lazarus, 2016). Patient anti-HLA antibody testing to detect antibodies directed against HLA of the available UCB unit using standardised methodology might be included in the algorithm of UCB selection. In general, the use of UCB units with donor specific antibodies is not advisable due to the increased risk of graft failure and transplant related mortality (Cutler et al., 2011), (Mary Eapen et al., 2011), (Annalisa Ruggeri et al., 2013).

The significance of recipient-UCB ABO group mismatch has not been fully explored. However, some groups described decreased OS and disease-free survival rates in UCBTs for adults with haematological malignancies in cases where an ABO major incompatibility was present (Arcese et al., 2006). Recent study on double cord blood unit transplantation for adults with haematological malignancies revealed the importance of ABO compatible CBUs on improved overall survival post UCBT (Vanderson et al., 2015).

1.4.4. *UCB unit cryopreservation time and transplant outcomes*

The first CBBs were established in the early 1990s. Umbilical cord blood unit can be collected and cryopreserved for years. Storage of UCB units comes at a financial cost to cord blood banks, which is ultimately passed on to the transplantation cost to the transplant centres. Another important factor is the impact of long-term cryopreservation on transplants outcomes. The impact of

cryopreservation time on progenitor cell function has been addressed. It was demonstrated that UCB units stored for up to 20 years do not lose function when used *in vitro* and in murine assays of progenitor cell function (Broxmeyer et al., 2003). In 2015, *Mitchell et al* analysed 288 single UCBs units used for the transplantation from 1992 to 2013, with duration of cryopreservation ranging from 0.8-11.07 years. The group established that “the number of years the UCB unit spent in cryopreservation had no impact on TNC recovery or UCB post-thaw viability. Duration of cryopreservation also had no impact on neutrophil or platelet engraftment in single UCB transplantations” (Mitchell et al., 2015). The authors admitted that one of the limitations of the study was presence of only few units that had been cryopreserved for > 10 years. In summary, results of the study were reassuring and supported the idea that UCB units can be clinically used even after long-term cryopreservation.

The Eurocord group study assessed 1351 UCBT recipients given single-unit UCBT following a 57yeloablative conditioning regimen and found that storage duration was not associated with neutrophil recovery or survival (median storage time was 2.3 years, range 0.3-14) (Rocha & Gluckman, 2009).

1.5. The role of HLA in UCBT

1.5.1. *Introduction*

Alloantigens can be divided into major (MHC) and minor (MiHA) histocompatibility complex antigens (Hirayama & Azuma, 2011). The MHC is referred to as the HLA complex in humans. The genes related to the HLA system encode a complex array of histocompatibility molecules which play a central role in immune responsiveness and determining the outcome of HSCT in humans (Hirayama et al., 2012). “The primary goal of histocompatibility testing for patients who are undergoing HSCT is the identification of a suitable HLA-matched donor to reduce the risk of post-transplant complications, which may result from HLA incompatibility” (Hirayama & Azuma, 2011). In the UK, a fully matched unrelated donor can be identified for approximately 70% of patients of white Northern European descent but only 20% of non-white Northern European descent (S. G. Marsh et al., 2013), (Hough et al., 2016). The availability of mismatched unrelated donors, haploidentical and cord blood donors has increased access to transplant for people from ethnic minority groups. This is particularly the case with UCBT, where less stringent patient-UCB HLA matching is permitted (Ballen et al., 2013).

1.5.2. *UCB-patient HLA-matching and the impact on transplant outcome.*

The effect of HLA mismatches after bone marrow transplantation from unrelated donors has been well studied (Atsuta et al., 2013). “Four loci, HLA-A, -B, -C and

HLA-DRB1 allelic level matched (i.e. 8/8) to the patient bone marrow is currently the first alternative if a suitable HLA-identical sibling donor is not available” (Atsuta et al., 2013). An increase in the number of donor-recipient allelic level HLA mismatches at four loci from 8/8 to 7/8 or less is associated with higher mortality, with approximately 10% reduction in OS in unrelated bone marrow transplantation (Atsuta et al., 2013), (Lee et al., 2007). The first UCBT was performed using UCB from an HLA-matched sibling. Subsequent studies have shown that UCBT is permissive at one or two HLA mismatches, “since HLA mismatches are better tolerated after UCBT with lower incidence of severe GvHD” (Mary Eapen et al., 2007),(Wagner et al., 2002)(Barker, Scaradavou, & Stevens, 2010).

Traditionally, UCB units had been matched to the patient for HLA-A and –B antigens, and HLA-DRB1 alleles (Ballen & Lazarus, 2016). With these criteria, HLA matching for UCB transplantation is less stringent compared with transplantation with BM or PBSC because these treatment methods require at least high resolution HLA-A, -B, -C and –DRB1 matching for optimal transplant outcome, as mentioned above. “This less stringent HLA matching of UCB transplantation extends the available donor pool and increases the probability of finding a suitable donor, especially for patients with rare HLA alleles” (Barker, Byam, et al., 2010). Recently, the importance of HLA-C matching on UCBT transplant outcome has been established. In the case of adult unrelated HSCT, several studies have reported more acute GvHD and higher mortality after transplantation mismatched at HLA-C (Lee et al., 2007) (Bray et al., 2008). “Matching at HLA-C antigen, at least for myeloablative conditioning in acute

haematological malignancies and single-unit UCBT also has been shown to improve survival” (Mary Eapen et al., 2011). Recently, the importance of allelic-level UCB-recipient matching in single-unit UCB selection has been proposed. Patients who were transplanted with units matched HLA-A, -B, -C and –DRB1 alleles had a lower TRM than patients with one or more allelic level mismatches at these loci in a myeloablative UCBT setting for haematological malignancies (Mary Eapen et al., 2014). The impact of patient-UCB allelic level HLA matching in the case of double-unit UCBT is still under investigation. The reports on this topic are controversial, favouring a greater or lesser degree of mismatching. In 2015, *Oran et al* published data on 133 predominantly adult patients with haematological malignancies who underwent a double-unit UCBT. They showed substantial difference in 2-year TRM in the 7-8/8 matched group in comparison to the 5-6/8 and 4/8 or less matched groups (0% versus 39% versus 60% respectively) (Oran et al., 2015). A recent report by *Brunstein et al*, showed that in a cohort of 342 patients with haematological malignancies (predominantly adults), a greater level of allelic level HLA-mismatching between recipient and UCB graft did not negatively impact on the incidence of engraftment, rate of acute and chronic GvHD or non-relapse mortality. Furthermore, greater HLA-mismatching protected against leukemic relapse in a subset of 174 patients (Brunstein et al., 2015).

There is a strong relationship between HLA-mismatch and cell dose in UCBT. In 2009, a *Eurocord* study published data suggesting that HLA disparity might be abrogate by increasing the number of TNCs in the UCB graft. They recommended a higher dose of TNC if the single-unit UCB graft is only 4/6 HLA

matched (“TNC > 3.5×10^7 /kg upon freezing and $>2.5 \times 10^7$ /kg on thawing for malignant disorders; TNC > 4.0×10^7 /kg upon freezing and $>3.5 \times 10^7$ /kg on thawing for non-malignant disorders”) (Rocha & Gluckman, 2009). In line with the above study, *Baker et al* in 2010, showed that in 1061 UCB recipients with haematological malignancies, “a better HLA-match compensated for lower TNC dose” (Barker, Scaradavou, et al., 2010a).

In the case of single-unit UCBT, BSBMT CBWG guidelines take into consideration both HLA matching (recommended at allelic level for all four loci) and cell dose of the UCB. In the case of a single locus mismatched UCB, avoidance of –DRB1 mismatch is recommended. In the case of 4-7/8 matched units, TNC dose should be as high as $> 5.0 \times 10^7$ /kg of recipient’s weight. CBUs which are matched at 3/8 are not recommended for transplant. In the case of double-unit UCBT, there are still insufficient data and “the role of high resolution typing is not yet defined” (Hough et al., 2016). HLA matching between the two units in a double CBU transplant is not required (Hough et al., 2015).

1.6. Immune reconstitution post UCBT

“Despite considerable progress in the management of HSCT complications, infections remain an important cause of post-transplant morbidity and mortality. The major advance in the management of infectious complications has come from a better understanding of the mechanisms of the complex depression of immunity observed during the first months after HSCT.” (Sauter, Barker, et al., 2011). “Reconstitution of T-cells post HSCT occurs in 2 phases” (Grewal et al.,

2003) . In the first months post HSCT, the recipient's defence mainly consists of peripheral proliferation of memory (previously antigen-exposed) T-cells, either infused with the graft or residual host T-cells which escaped pre-transplant conditioning. This response is restricted to the T-cell population with limited T-cell receptors (TCR) and also occurs faster in CD8+ compared to CD4+ T-cells (Grewal et al., 2003),(Seggewiss & Einsele, 2010). "Later, thymus-dependent proliferation of T-cells derived from the graft occurs" (Grewal et al., 2003), (*EBMT-ESH handbook 6th Edition, 2012*). This pathway is a much more prolonged process and may be completed as late as 8-12 months after HSCT (Danby & Rocha, 2014). This pathway may be impaired by the age-dependent involution of the thymus, GvHD and the conditioning regimen itself (Danby & Rocha, 2014), (Barrett, 2008).

Immune reconstitution after UCBT has always been considered slower, compared to BM or PBSC grafts, owing to the naïve nature (antigen – inexperienced) of infused T-cells, lower cell dose, low T-cell levels of activation markers and reduced levels of cytokines produced in response to antigens (Szabolcs & Niedzwiecki, 2007), (Remberger, Persson, Mattsson, Gustafsson, & Uhlin, 2012). The majority of the studies have reported delayed immune reconstitution after UCBT when serotherapy (in particular ATG) was used in order to prevent graft rejection. This resulted in a higher risk of opportunistic infections. Early comparative studies on graft sources showed that "the risk of bacterial and viral infections was higher after UCBT than after a full matched or mismatched unrelated donor transplant" (Ballen et al., 2014). For these reasons, many centres have abandoned the use of ATG resulting in better

immune reconstitution without increasing the rates of rejection or acute GvHD (Boiron et al., 1998) (Mohty & Gaugler, 2010). *Sauter et al.*, in 2011 published data on rapid (within 4 month) immune reconstitution after double-unit UCBT with no serotherapy in older patients (Sauter, 2011). However, recently published laboratory data reports refuted this. There is a suggestion that fetal and adult T-cells are different. The majority of UCB T-cells have a naïve (CD4+CD45RA+) antigen phenotype. Despite this, it was demonstrated that under stimulation, these naïve T-cells can transform more quickly than adult T-cells into functionally active central memory (CD4+CD45RO+) T-cells *in vitro* (Early & Reen, 1999). Interestingly, *Zhang et al* in 2014, demonstrated a presence in UCB cell populations of fetally developed CD4+ T-cells with an effector memory phenotype (CD4+RO+) that displayed a large variety of inflammatory effector functions (Zhang et al., 2014). Later, these data were complimented by clinical observations. *Talvensaari et al.*, in 2002, compared data from paediatric patients (with malignant disorders) who received either a UCBT or matched sibling donor transplant and demonstrated that UCB recipients had higher naïve CD4+ T-cells and T- cell receptor (TCR) diversity (Talvensaari et al., 2002). In 2012, *Chiesa et al* showed “ early (within 2 months) thymic-independent peripheral CD4+T-cell expansion, with a rapid shift from naïve to central memory phenotype and early regulatory T-cell recovery”, in a group of 30 paediatric recipients of T-cell replete UCBT for malignant and non-malignant disorders. Both viral infections and acute GvHD were frequent, but well controlled (Chiesa et al., 2012). Furthermore, the ability to generate virus-specific T-cells that target multiple viruses such as CMV, adenovirus and EBV

from naïve T-cell populations in UCB, confirms the ability of these cells to function (Hanley, Bollard, & Brunstein, 2015).

No major differences have been shown with regards to numbers and distribution of NK cells in CBU and adult peripheral blood. Moreover, even more NK cell precursors were described in UCB (Gaddy & Broxmeyer, 1997) with more rapid and sustained expansion (Charrier et al., 2013).

“The immune response of the graft can also result in a GvL effect, potentially decreasing the risk of relapse in certain haematological malignancies” (Bleakley & Riddell, 2004) (Barrett, 2008). The functional naïvity of neonatal lymphocytes raised concern for a reduced GvL effect, but clinical experience with UCBT showed that disease status (i.e. remission *versus* advanced disease) at HSCT still remains the most important factor in predicting outcome (Wagner et al., 2002) (F Locatelli et al., 1999). Moreover, a report by *Hiwarkar et al* in 2015, demonstrated that UCB T-cells provided a more potent anti-tumour effect than adult T-cells in a B-cell lymphoma mouse model, through enhanced recruitment of naïve UCB T-cells, prompt induction of memory effector differentiation and gaining of cytotoxic effector functions in the tumour microenvironment (Hiwarkar et al., 2015).

1.7. Conclusion

In the last few decades of UCBT history, scientists and clinicians have accumulated an enormous amount of knowledge and understanding of the kinetics of hematopoietic recovery post UCBT. This includes: “a minimum safe transplantable dose, quality and characterisation of the UCB inventory; use of double-unit CBU for infusion and methods for *ex vivo* expansion of UCB” (M E Horwitz, 2014). UCB graft selection is currently based on HLA matching and cell dose. “There is a recognised complex interaction between cell dose and HLA type” (Ballen & Lazarus, 2016). Although previously it was considered that less stringent HLA matching was acceptable in the UCBT setting; however, new data have emerged suggesting that a more precise HLA-matching approach might improve transplant outcome. Both NIMA and IPA matching also appear to play a role in UCBT outcome. If such matching can be implemented in the routine UCB selection algorithm, it will give a chance for more patients in need of a transplant to find a suitable donor.

Limitation of cell dose can be overcome by, either performing double-unit UCBT or expanding single UCB grafts. As mentioned above, double-unit UCBT for paediatric patients with haematological malignancies does not provide an additional beneficial effect in terms of relapse incidence and transplant-related mortality. However, double cord allows safe transplantation in larger adolescence and adults for whom an adequate single unit could not be found. Cord blood unit expansion showed promising short-term outcomes, but longer follow-up is needed. In addition, the

expansion techniques require specialised laboratories and are expensive. A more cost-effective option to solve the problem of low cell dose in UCB would be to optimise UCB collection techniques based on the knowledge of which maternal and delivery factors may have an impact on cell count. In the next chapters of my thesis, I will be looking at the impact of delivery mode, previous history of maternal infections, CMV status and numbers of previous pregnancies on the collected UCB cell count and UBCT outcomes.

Chapter 2. Delivery mode influence on the volume and total nucleated cell count of the UCB

2.1. Introduction

From the time the first cord blood transplant was carried out in 1988, Cord Blood Banks (CBB) have been established across the globe to collect and cryopreserve the cord blood for allo-HSCT. The ever increasing number of banked UCBs has underlined the need for cost-efficient management of the CBBs (Page et al., 2014). According to the WMDA CBB activity report from 2012, UCBs with TNC of 200×10^7 have a 5.63% yearly likelihood of being used. By contrast, units with TNC below 90×10^7 have a probability of use of 0.08% per year (www.wmda.info).

In the introduction to the thesis, I described the numerous UCB qualitative and quantitative factors that are believed to influence UCBT outcomes. For this reason, CBBs strive to increase the quality of inventories typically through stringent selection from collected UCBs. Different CBB employ different techniques for UCB collection.

Typically, UCB may be obtained from vaginal deliveries and/or after Caesarean section, before or after the placental detachment (i.e. *in utero* or *ex utero*). Several obstetric, maternal and fetal factors (i.e. mode of delivery, maternal

age, maternal ethnicity, birth weight) will make an impact on the quality of collected CBU (Shlebak et al., 1998), (Lim et al., 2000), (Jones et al., 2003). Maternal and obstetric factors, influencing qualitative and quantitative parameters of collected UCB are outlined in Table 2-1.

Table 2-1: Maternal and obstetric factors influence on the quality of UCB.

Maternal and obstetric factors	Fetal factors	Reference
-Better CFU in NVD > CFU in IVD and el/em CS -Term pregnancies → higher TNC count and larger V	Larger V collected from infants with BW ≥ 2.5 kg	(Shlebak et al., 1998)
Prolonged first stage of labour → higher TNC and CD34+ count	Low umbilical venous blood Ph → higher NC and CD34+ count	(Lim et al., 2000)
Prolonged labour and em/el CS → larger V		(Jones et al., 2003)
-Placenta's weight > 695g → larger V and higher TNC count -Term pregnancies → higher TNC count -Prolonged labour → higher TNC count	Infants with BW ≥ 3.150 kg → larger V collected	(Solves et al., 2005)

<p>-Placenta's weight > 600 g → larger V and TNC count</p> <p>-Gestational age > 39 weeks → higher CD34+ count</p> <p>- em/el CS → larger V but lower TNC count</p>	<p>Bigger infants (no weight specified) → larger V and higher TNC and CD34+ count and CFU</p>	<p>(Mancinelli et al., 2006)</p>
<p>-em/el CS → larger V and higher CD34+ count</p> <p>- IVD/NVD → higher MNC</p>		<p>(Omori et al., 2010)</p>
	<p>High umbilical arterial pCO₂ and low pH → higher MNC</p>	<p>(Ebina et al., 2012)</p>
<p>Abbreviations: BW- body weight; UCB- umbilical cord blood; CFU- colony forming unit; emCS- emergency Caesarean section; elCS- elective Caesarean section; IVD- instrumental vaginal delivery; NVD- normal vaginal delivery; MNC- mononuclear cell count (x10⁸); TNC- total nucleated cell count (x10⁷); V- volume (ml)</p>		

To address the question of practice in UCB collection at Anthony Nolan Cell Therapy Center (CTC) a retrospective study on impact of the mode of the delivery on the quality indicators of UCB (i.e. volume and TNC) was conducted.

2.2. Materials and methods

Between August 2012 and November 2013, 8,346 UCB were collected at four designated UCB collection centers (Royal Free London NHS Foundation trust, University Hospitals of Leicester NHS Trust, University Hospital Birmingham

NHS Foundation Trust, and Kings College Hospital NHS Foundation Trust). Of these, 2,411 UCBs met clinical criteria to be included in the clinical inventory of the Anthony Nolan CTC (i.e. time from collection to reception < 32 hours, volume > 85ml, TNC > 14×10^8 , CD34 > 3.2×10^6).

Collections were performed after normal vaginal delivery (NVD), instrumental (forceps or ventouse) vaginal delivery and Caesarean (elective and emergency) deliveries, by a skilled technician using an *ex utero* method of collection in line with the Royal College of Obstetricians and Gynecologists (RCOG) and Royal College of Midwives (RCM) recommendations, which support the practice of deferred cord clamping (RCOG, 2009). UCBs were transported from the collection centers to the CTC and processed within 24.6 hours on average.

Results are expressed as mean \pm standard deviation (SD). Comparisons between groups were performed using the two-way t- test and p- values of less than 0.05 were considered to be significant. Graph represents the percentage number of units considered as clinical, per group, according to the thresholds detailed in the results section.

2.3. Results

2.3.1. Collection mode

In the cohort of 3,789 collected UCBs which met the volume threshold and so were subsequently analysed for cell content, most of the units were collected after CS (46%), of which 32% were elective CS. Normal vaginal deliveries (37%) were the second more frequently used collection type.

2.3.2. UCB volume

The volume of UCBs collected from CS was greater than in vaginal deliveries (119.55 (± 26.52), 110.46 (± 23.04) respectively, ($p=0.04$). There were no differences in the volume of UCB, when CS group was subdivided into eICS and emCS, ($p>0.05$).

2.3.3. UCB TNC

In the CS group, the number of TNC was higher in the UCBs collected from the emCS ($168.4 \pm 80.5 \times 10^7$) when compared to the group of eICS ($131.72 \pm 63.4 \times 10^7$), ($p=0.03$). However, UCBs collected from the CS showed the lowest TNC number ($142.9 \pm 71.1 \times 10^7$) in contrast to IVD ($184.5 \pm 79.0 \times 10^7$), ($p=0.03$).

Therefore, highest TNC was observed from the UCBs collected from the IVD ($184.5 \pm 79.0 \times 10^7$), ($p=0.02$). A comparison of collected UCB parameters

(volume and TNC) obtained from different modes of deliveries is displayed in Table 2-2.

Table 2-2 : Collected UCBs parameters (volume and TNC)

Type of Collection	% (N)	Average Volume (ml)	Average TNC ($\times 10^7$)
Elective Caesarean section	32% (1231)	120.03 \pm 26.85	131.72 \pm 63.43
Emergency Caesarean section	14% (547)	118.47 \pm 25.76	168.14 \pm 80.56
Instrumental vaginal delivery	16% (625)	112.46 \pm 23.59	184.47 \pm 79.02
Normal vaginal delivery	37% (1386)	109.28 \pm 22.63	152.27 \pm 66.47

The TNC number was higher in UCB collected from instrumental vaginal deliveries and emCS whereas volume of the CBU was much less from the same mode of the delivery.

2.3.4. Mode of the delivery and UCBs stored for clinical use

There were more CBU collected from the IVD (68.9%) and emCS (53.7%), which were suitable for clinical use. Please see Figure 2. 1

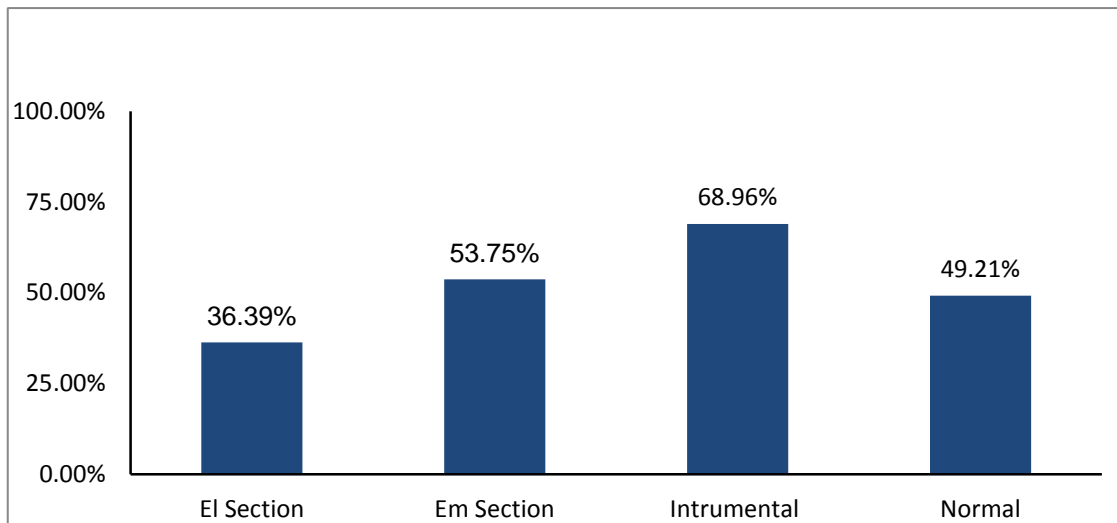


Figure 2-1 : Clinical conversion rates with breakdown of collection type

CBU collected from the instrumental vaginal delivery in 68% met the criteria for the clinical use. In contrast, only 35% CBU collected from emCS group made it for the clinical use.

2.4. Discussion

During fetal development, the concentration of hematopoietic stem cells in circulation usually remains stable. However, an increase in concentration can be observed shortly before delivery. This increase in concentration declines rapidly after birth (Ary L Aughlin et al., 2001). The normal birth process is a stress itself and if prolonged, is associated with even increased delivery stress. Usually, in response to any stress, levels of cytokines and hormones increase. In relation to the birth process, the strongest positive correlations were found between UCB progenitor cells number and maternal serum IL-4, IL-10 and GM-CSF levels (Juutistenaho, Eskola, Sainio, Aranko, & Kekomäki, 2010). UCB cortisol level as a marker of fetal stress, was demonstrated to be higher in infants born by forceps vaginal deliveries than those born by ventouse vaginal deliveries (Gitau et al., 2001). In addition, under stress, numbers of nucleated cells in the cord bloods increase as a result of demargination of cells from the vascular endothelium mediated by catecholamines (Solves et al., 2005), (Lim et al., 2000). Gestational age is an important factor too. The number of circulating CD34+ cells is significantly higher in pre-term infants when compared with full-term newborns (Gonzalez et al., 2009). In addition, cord clumping itself, following by oxygen deprivation may play an additional stimulating role in stem cell mobilisation (Gonzalez et al., 2009).

Different delivery modes are associated with different levels and types of stress factors on the mother and infant. Therefore this might potentially influence the quality parameters of the obtained UCB.

In this study, more TNC were obtained after the IVD ($184.47 \pm 79.02 \times 10^7$) ($p=0.02$) and emCS ($168.14 \pm 80.56 \times 10^7$), ($p=0.03$). Both modes of the delivery are certainly an additional stress factors for both mother and baby. Within the CS group, higher TNC number was observed in the group of the emCS, rather than in eICS ($168.14 \pm 80.56 \times 10^7$, $131.72 \pm 63.43 \times 10^7$ respectively), ($p=0.03$) Within the VD group, more TNC were obtained from IVD ($184.47 \pm 79.02 \times 10^7$) rather than in NVD ($152.27 \pm 66.47 \times 10^7$), ($p=0.04$). Vaginal deliveries (instrumental or spontaneous) are more likely to be happening from term pregnancies when baby's weight is usually higher than in babies born pre-term. Higher neonatal weight was reported to correlate with higher TNC (Mancinelli et al., 2006). In addition, neonates with higher weight are more likely to require assistance during the obstetric process (cephalo-pelvic disproportion, i.e. discordance between size of the babies' head and the birth canal) that might cause increase in the release of stress cytokines. Those two factors- stress and infant weight, might explain the higher TNC in UCB obtained from IVD.

Consistent with the previous studies, the volume of UCB collected from the both eICS (120.03 ± 26.85 ml) and emCS (118.47 ± 25.76 ml) were higher than the volume of the units obtained from both NVD (109.28 ± 22.63 ml) and IVD (112.46 ± 23.59 ml) deliveries (Jones et al., 2003),(Omori et al., 2010). "Placing the neonate on the maternal abdomen (above the level of the placenta) after the delivery prior to clamping the cord increased the volume of the CBU" (Grisaru et al., 1999). "The flow of the blood into the placental cord is assisted through this method. As per another hypothesis the possibility of blood clot

formation is minimised after a C-Section, where manual removal of placenta happens quicker as compared to vaginal delivery” (Mancinelli et al., 2006).

This study included significant amount of the UCB collected via el/emCS (46%). It showed that bigger volume of the collected UCB did not necessarily result in a higher TNC. In our collected UCBs, the TNC number was higher in UCBs collected from instrumental vaginal deliveries and emCS whereas volume of the UCBs was much less from the same mode of the delivery.

In daily practice, it is relatively easy to collect UCB in eICS (i.e. easy to consent, planned procedure). However, this study has shown that only 36% UCBs collected from eICS group were clinical grade (due to the low TNC number). In contrast, 68% of the UCBs collected from the instrumental vaginal delivery met the criteria for the clinical use.

For decades, timing of cord clumping (immediate *versus* delayed) has been under the continuous discussions. Historically, there was a concern, that immediate cord clumping (within the first 15 seconds) increases an infant’s risk for jaundice or polycythemia (YAO, 1969). However, in recent years, several randomized studies provided a very strong evidence that delayed cord clumping (at 2-3 minutes after the delivery) has no harmful effect on the infant (Kluckow & Hooper, 2015), (Stuart Hooper et al., 2016). On the other hand, in context of CBU collection, the immediate cord clumping results in more blood being transferred to the placenta with potentially more numbers of hematopoietic stem cells available for the collection (Tolosa et al., 2010). Nevertheless, the current guidelines on of the Royal College of Obstetricians and Gynecologists

recommend to avoid delayed cord blood clumping and therefore an immediate clumping is the standard practice in the UK CBUs collection centres (Rcog, 2009).

Cord blood banking is becoming increasingly expensive mainly due to the strict regulations required to guarantee the quality of the product. Therefore, cord blood collection procedures might contribute to reduce the cost of maintenance by means of storing only the high quality units that would have more chances to be utilized clinically. This study indicates that UCBs harvested after instrumental vaginal deliveries and emergency Caesarean section may provide optimal units for further stem cell transplantation.

Chapter 3. Donor-recipient allele-level HLA matching of unrelated cord blood units.

3.1. Introduction

Donor-recipient HLA matching remains the most significant factor in donor selection for HSCT. As mentioned previously, the selection of UCB was traditionally based on low/intermediate resolution typing for HLA Class I genes (-A and -B), and high resolution typing for HLA Class II genes (mainly -DRB1).

As a consequence, UCB units that are selected for transplantation frequently contain more mismatches when high resolution typed for all 4 loci (HLA-A, -B, -C, -DRB1). For example, in 2005 in a study of 122 UCB-recipient pairs, *Kogler et al* showed that “after high resolution typing for HLA-A , -B and -DRB1 loci and inclusion of HLA-C and HLA-DQB1, 14% of recipients were matched for 9-10/10 alleles, 63% were matched for 6-8/10 and 23% showed more mismatches” (Kögler, Enczmann, Rocha, Gluckman, & Wernet, 2005). The group did not evaluate transplant outcomes of the selected units because the sample size was too small. In 2008, Cord Blood Transplantation Study (COBLT) published results on 179 UCB-recipient pairs. “When typed for all 3 loci at high resolution (HLA-A, -B and -DRB1), about one third of the pairs were found to be more mismatched than what the initial low resolution typing had indicated” (Martin et al., 2006).

The impact of UCB-recipient high resolution matching on single and double unit UCBT outcome has been discussed in my literature review section. To summarise, increased UCB-recipient HLA disparity was associated with inferior neutrophil and platelet engraftment along with an enhanced risk of acute GvHD and transplant related mortality. “Increasing the TNC dose may ameliorate but not completely compensate the negative impact of HLA mismatch” (Barker, Scaradavou, & Stevens, 2010). UCB-recipient high resolution matching in the case of single unit UCBT is recommended due to the reported higher rates of overall survival in UCB-recipient pairs matched at high resolution. In the context of adult unrelated donor HSCT, the influence of single allelic level mismatches on transplant outcome is well described. Mismatches at HLA-A and –C are reported to be better tolerated than mismatches at HLA-B or –DRB1 (Lee et al., 2007). Data on “preferable” mismatches in the context of UCBT are different and still unclear. In 2011, in a study involving 803 recipients of single unit UCBT, *Eapen et al* showed for the first time that specific allelic mismatches, such as at HLA-C, should be avoided either as a single mismatch or in combination with other allelic mismatches (Mary Eapen et al., 2011). Later, in 2014, a joint CIBMTR and Eurocord study on the outcome of 1658 recipients of single unit UCBT was performed. It showed that a “single allelic mismatch at HLA-A, -C or –DRB1 was associated with increased non-relapse mortality” (Mary Eapen et al., 2014). However, in 2015, a Japanese group performed an analysis of single unit UCBT outcome on 107 adult recipients with malignant disorders with UCB-recipient matching at low/intermediate level. In multivariate analysis they demonstrated a reduced risk of relapse; and as a consequence, improved OS in recipients of mismatched grafts. However, the cumulative

incidence of grades II-IV acute GvHD was significantly associated with a mismatch at HLA-DRB1 ($p=0.03$). It may be deduced that the presence of acute GvHD is a beneficial factor of lower relapse rate due to the possible GvL effect (Konuma et al., 2015). The difference between the 2 studies (Eurocord versus Japan) is not clear. It might be explained by a difference in age group of selected recipients (i.e. predominantly children in the *Eurocord study*) or even different geographic location. Current UK consensus guidelines on UCB selection recommend avoiding HLA-DRB1 mismatch (Hough et al., 2015). For non-malignant conditions the data on HLA matching and interaction between the cell dose and HLA is not very apparent. However, it is generally recommended that better HLA matching and higher TNC dose should be considered (Rocha & Gluckman, 2009).

Given the current consensus on selecting cord blood units for transplantation based on allelic level HLA matching (at least in the case of single unit cases), I retrospectively evaluated the current practice of UCB selection by UK transplant centres. The analysis was focused on evaluation of the extent of HLA mismatch at high resolution (for HLA –A, –B, –C and –DRB1) between UCB-recipient pairs for the available cases (Figure S- 1, Appendix).

3.2. Materials and methods

Cord blood unit work-up requests for 208 recipients from 12 UK transplant centres were received by Anthony Nolan between 1st January 2013 and 31st December 2015. Selected UCB and recipient HLA-phenotypes were obtained from the Anthony Nolan database. UCB-recipient matching was determined at antigenic level (for HLA-A, -B antigens) and at allelic level (for HLA-DRB1 allele) and at allelic level (for HLA-A, -B, -C and –DRB1) (Table S- 2Table S- 2 Appendix). In cases where high resolution typing of cord blood unit and/or recipient was not performed, HLA matching was predicted based on published HLA haplotype frequencies (S. G. E. Marsh, Parham, & Barber, 2000) .

3.3. Results

3.3.1. Characteristics of recipients

Recipient characteristics are described in Table 3-1. In total, 208 recipients (66% adults and 44% children) from 12 UK transplant centres were considered for single or double unit UCBT. Median age of recipients was 33 years (range, 5 months to 68 years). In total, 95 recipients (46%) were considered for single unit UCBT and 113 recipients (54%) were considered for double unit UCBT. Most recipients were undergoing UCBT for treatment of acute myeloid (47%) or lymphoblastic (11%) leukaemia. Other common indications for UCBT were: inborn errors of metabolism

(10%), myelodysplastic and myeloproliferative syndrome (9%) and lymphoma (8%).

Table 3-1 : Patients Characteristics

Characteristics	N (%)
Age	
≤ 18 y	92 (44%)
≥ 18 y	116 (66%)
Diagnosis	
AML	97 (47%)
ALL	24 (11%)
IEM	20 (10%)
MDS/MPS	18 (9%)
Lymphoma	16 (8%)
ID	14 (7%)
CML/CLL	9 (4%)
BMF	5 (2%)
Other	5 (2%)
Multiple myeloma	3
Immune enteropathy	1
Not known	1
<p>ALL indicates acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; IEM, inborn errors of metabolism; MDS, myelodysplastic syndrome; MPS, myeloproliferative syndrome; ID, immunodeficiency; CML, chronic myeloid leukaemia; CLL, chronic lymphocytic leukaemia; BMF, bone marrow failure syndrome; MM, multiple myeloma.</p>	

3.3.2. Extent of UCB-recipient pair HLA mismatch at low/intermediate resolution

In total, 321 cord blood units were evaluated: 95 units were used as single unit grafts and 226 units were used as double unit grafts. Median cryopreserved TNC dose was $2.9 \times 10^7/\text{kg}$ recipient's body weight (range: 1.26 to $41.8 \times 10^7/\text{kg}$).

At 'classic level' of matching (at antigen level for HLA-A and -B and at allelic level for HLA-DRB1), most of the cord blood units were 4/6 (n=152, 47%) or 5/6 (n=130, 40%) HLA matched to the recipient. Only 13 % (n=38) of the selected units were 6/6 HLA matched to the recipient. One unit was 3/6 HLA matched to the recipient and was considered for double unit UCBT. The distribution of low/intermediate level UCB-recipient HLA match is shown in Figure 3-1: Distribution of low/intermediate level umbilical cord blood unit-recipient HLA match (Figure 3-1)

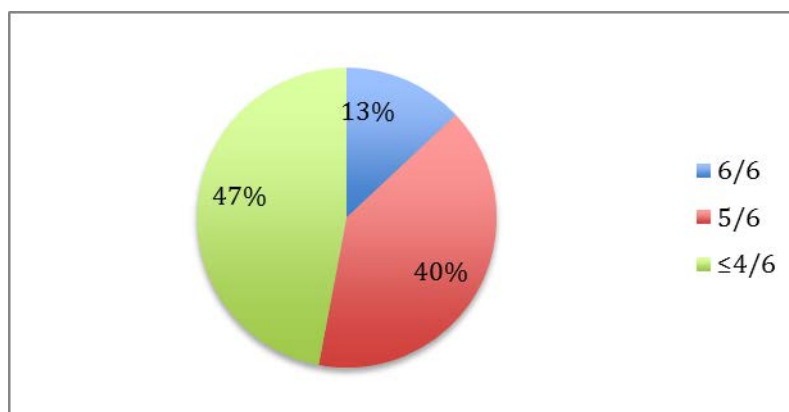


Figure 3-1: Distribution of low/intermediate level umbilical cord blood unit-recipient HLA match

In the 6/6 UCB-recipient HLA matching group, most units (i.e. 31/38, 82%) were considered for single unit UCBT. In contrast, most of the units in the 4/6 UCB-recipient HLA matching group were considered for double unit UCBT (140/152, 92%).

3.3.3. Extent of UCB-recipient HLA mismatch at high resolution.

For all cases patient/UCB allelic level match was evaluated.

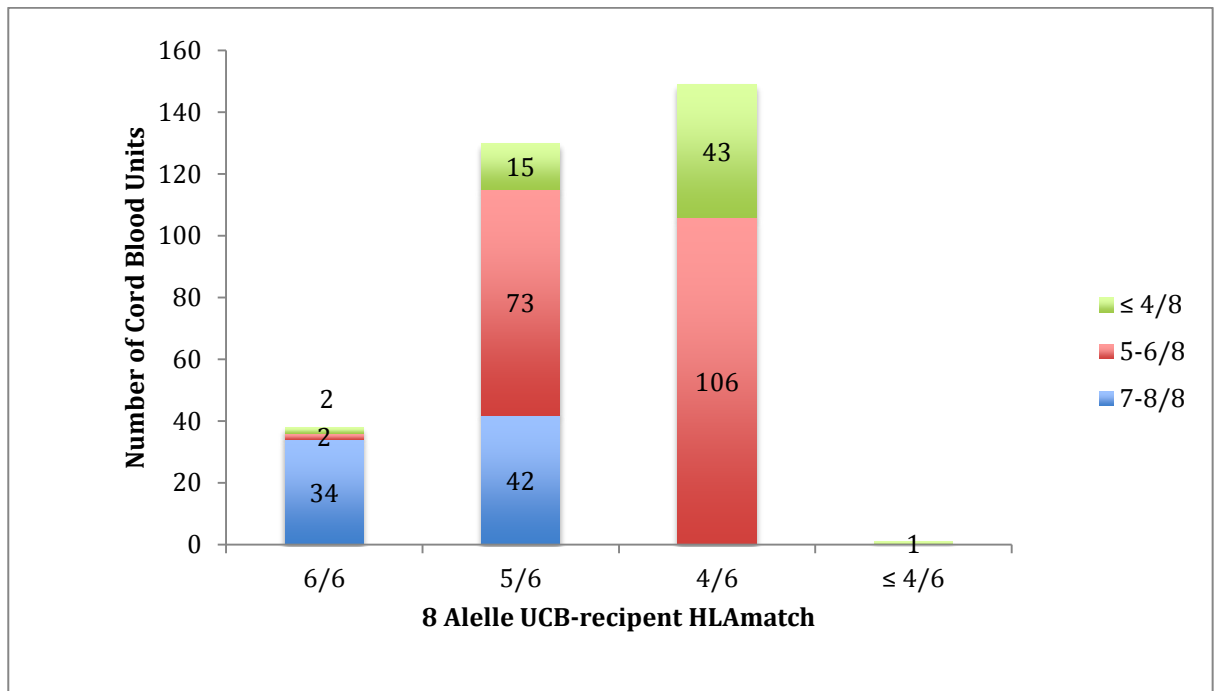
Almost all cord blood units within the 6/6 UCB-recipient HLA matched group were found to be matched at 7-8/8 with the recipient at high resolution (34/38, 89%). However, 2/38 units from that group were found to be \leq 4/8 HLA matched with the recipient. These 2 units were considered for double unit UCBT since cord blood unit allelic matching with the recipient on HLA Class I is not recommended at present for the double-unit UCBT (Hough et al., 2015). The remaining 2 units were 5-6/8 matched with the recipient.

Cord blood units from the 5/6 UCB-recipient HLA matched group in most cases appeared to be 5-6/8 HLA matched with the recipient when typed to high resolution (73/130, 56%). In 11% (15/130) of cases, the units were found to be \leq 4/8 HLA matched with the recipient when typed to high resolution. In 2 cases from that group, the units were considered for single unit UCBT, where \leq 4/8 UCB- recipient HLA match is not recommended (Hough et al., 2015). The remaining units from 5/6 UCB-

Donor-recipient allele-level HLA matching of unrelated cord blood units.

recipient HLA matched group were 7-8/8 HLA matched with the recipient (42/130, 33%).

In the 4/6 UCB- recipient HLA matched group, none of the units were found to be 7-8/8 HLA matched to the recipient when typed to high resolution. Most of the units were 5-6/8 matched with the recipient (106/152, 70%). The remaining cases were $\leq 4/8$ HLA matched with the recipient (43/152, 30%). In most of these cases (44/46, 96%) the units were considered for double unit UCBT. However, in 2 cases, the units were used for single unit UCBT, where $\leq 4/8$ CBU-recipient HLA match is not recommended (Hough et al., 2015). Comparison of patient/UCB 4-loci allelic level matching and “classic” level of matching is shown in Figure 3-2



	7-8/8	5-6/8	$\leq 4/8$
6/6	89.5%	5.3%	5.2%
5/6	32.3%	56.2%	11.5%
4/6		70 %	30%
$\leq 4/6$			

Figure 3-2: Comparison of patient/UCB 4-loci allelic level matching and “classic” level of matching

3.3.4. UCB-recipient HLA allelic match and TNC dose

I evaluated both UCB-recipient HLA allelic match and TNC dose of the selected unit. The indication for UCBT (malignant versus non-malignant disorder) in each case was taken into account.

In all but one of the cases, TNC dose per recipient’s weight was in accordance with the current UK guidelines (Hough et al., 2015). In one case, TNC dose was less than currently recommended for <8/8 HLA matched units (i.e. $<5 \times 10^7$ /kg recipient’s weight for a non-malignant disorder).

3.4. Discussion

Selection of umbilical cord blood units for transplantation based on HLA allelic matching is not well established. In the present study I demonstrated that UCB units routinely chosen for transplantation by UK transplant centres are selected based mainly on UCB-recipient HLA-A and –B antigenic and –DRB1 allelic level in determining the degree of matching. Some units showed substantial HLA disparity with the recipient when typed to high resolution for HLA-A, -B, -C and –DRB1, with units as low as 3/8 HLA allelic match being used for transplant.

Adoption of higher HLA matching criteria revealed that as many as 30% (46/152) of the cord blood units which were double HLA mismatched (i.e. 4/6) with the recipient at low/intermediate level, appeared to be \leq 4/8 HLA match at allelic level, which in general is not recommended, especially in the case of single unit UCBT. In 5% (5/95) of single unit UCBT cases, selection of the cord blood unit could have been changed. Four of these units showed \leq 4/8 UCB-recipient HLA matching at allelic level, which in general should be avoided. However, in all of these units, TNC dose was acceptable and in accordance with the current UK recommendations for that degree of mismatch (i.e. $>5 \times 10^7/\text{kg}$) (Hough et al., 2015). But in 1/5 cases, the cord blood unit used was 5/8 HLA matched with the recipient at allelic level and TNC dose was below the recommended standard (i.e. $< 5 \times 10^7/\text{kg}$ of recipient's weight for non-malignant condition).

In addition, 10% (4/38) of units which were considered to be a "perfect" HLA match with the recipient at low/intermediate level (i.e. 6/6), appeared to be \leq 6/8 HLA matched at allelic level. However, 89% (34/38) of these units were 7-8/8 HLA matched with the recipient at allelic level.

My findings have practical implications for the selection of cord blood unit, especially in a single unit UCBT setting. The transplant centres should be aware of the possibility of choosing sub-optimal cord blood units for transplantation when applying HLA matching at low/intermediate resolution. This is particularly important when selecting the cord blood unit for a single unit UCBT and from the 4/6 HLA matched group. Adoption of higher HLA matching criteria is

possible and may impact the selection of the “optimal” graft. Moreover, in my study I found that this could be achieved without compromising TNC dose. The lower limit of acceptable HLA allele mismatch also requires further investigation. The effect of allelic level HLA mismatch at different loci is also not completely understood and results reported by different groups are sometimes controversial. Unit selection becomes even more complex when TNC and CD34+ dose are taken into account. The introduction of high resolution UCB-recipient HLA matching in the double unit UCBT setting is still under discussion. Due to the small sample size I did not evaluate transplant outcome of the selected units in this series.

To summarise, as a result of improvements in HLA methodologies, some cord blood banks are now typing UCBs to high resolution for HLA-A, -B, -C and – DRB1. Third Generation Sequencing will deliver allelic level typing on all future UCBs stored by Anthony Nolan. Future UCB selection will be greatly supported by this information.

Chapter 4. Relationship between donor mothers previous exposure to the viral infections and UCBs cell count.

4.1. Introduction

In order to improve the chances of survival of the allogeneic foetus the mother's immune system undergoes changes. This also enhances her ability to respond to alloantigen's, especially with respect to infections. One of the most common of these infections is the Human Cytomegalovirus infection (CMV), which infects many people without producing overt symptoms. The frequency of CMV infection depends on age, geographical and socio-economic status (La Rosa & Diamond, 2012),(Griffiths et al., 2015). Typically, people born into poor socio-economic environments acquire CMV earlier than others. Antibody of IgG class, is a result of past infection and is present in approximately 60% of adults in developed countries; whereas in developing countries 100% adults have these antibodies present (Griffiths et al., 2015). Seropositivity was reported in over 90% amongst women of reproductive age in developing countries as opposed to western countries where it is less than 50% (Lissauer et al., 2011). "One of the unique features of CMV is the ability of persistence as a lifelong latent infection state in the host, particularly in the myeloid lineage cells, which can reactivate at any time" (La Rosa & Diamond, 2012).

Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

Active infection can be documented by the presence of IgM class antibodies and presence of the viraemia detected by the positive qualitative polymerase-chain reaction (PCR) for the CMV antigen. Several lines of studies have presented evidence that “innate immune responses are the first line of defence against the CMV in healthy individuals. In particular, NK cells are important in virus clearance and protection” (La Rosa & Diamond, 2012). It becomes extremely important in case of the viral infection in the perinatal period, when adaptive immune responses are not fully developed yet. At a later stage, the adoptive immune system takes over. CD8+ cytotoxic T-lymphocytes and CMV-specific helper CD4+ T-cells are considered the most important cells in controlling the virus.

In a study on a healthy adult individuals, primary CMV infection leads to a presence of a high percentage of virus-specific T-cells with recently activated naïve T-cells co-expressing CD45RA and CD45RO surface cell markers (La Rosa & Diamond, 2012). The first CD8+ T-cells detected express CD45RA-CD28-CD27+ phenotype following by resting CD8+ T-cells with memory phenotype (CD45RA-CD45RO+). “In the months following the primary infection, CMV-specific CD8+ T-cells gradually re-acquire CD45RA expression” (La Rosa & Diamond, 2012). Please see Figure 4-1.

Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

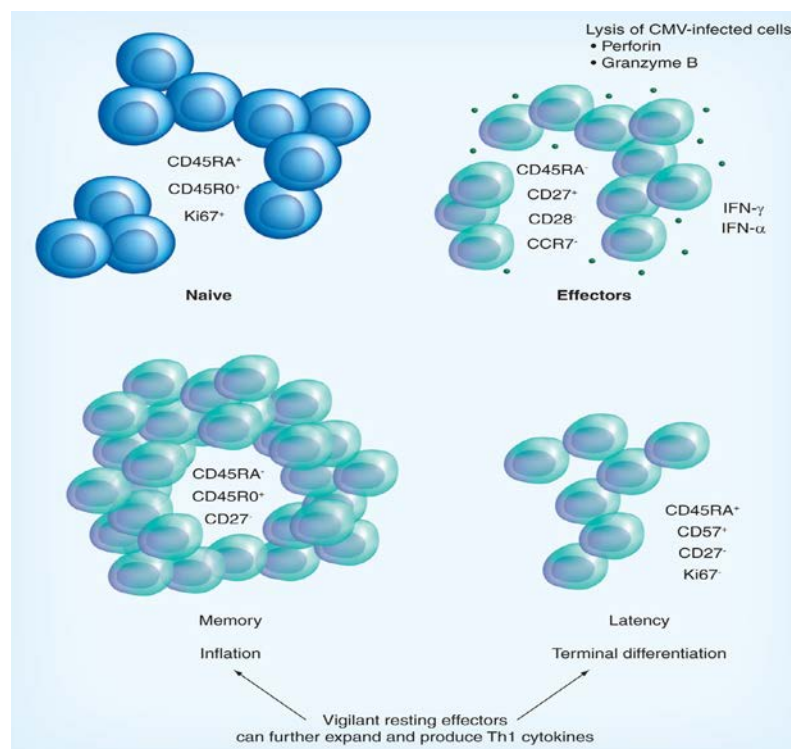


Figure 4-1: CMV-specific CD8+ T-cells phenotypes. La Rosa, C., & Diamond, D. J. (2012)

“The CMV genome contains approximately 200 open reading frames” (Lacey, Diamond, & Zaia, 2004). One of these is pp65 protein, which “has been most studied CMV protein and has been considered the predominant target of immune response against the virus” (“The Stealth Virus”: Prof Paul D Griffiths), (Solache et al. ,1999). After primary CMV infection, virus units are processed by antigen-presenting cells (APC), which stimulate the antigen-specific immune response (La Rosa & Diamond, 2012), (van de Berg et al., 2010). Establishment of the adaptive immune responses are crucial to prevent overwhelming CMV infection. CMV specific T-cells predominantly of a memory phenotype (both CD4+ and CD8+) are participating in this process (La Rosa & Diamond, 2012), (Sylwester et al., 2005).

Relationship between donor mothers previous exposure to the viral infections and UCBs cell count.

Congenital CMV infection causes major morbidity in newborn babies and affects up to 0.2-1% live births (“The Stealth Virus: Prof Paul D Griffiths: 9781477566794: Amazon.com: Books,” n.d.). The foetus can be infected when mother either contracts new infection or reactivates latent virus or gets reinfection with a new strain of the CMV (Griffiths et al., 2015). Among the women with primary CMV infection during pregnancy, 32% transmit virus across the placenta to produce intrauterine infection (Griffiths et al., 2015).

It is now recognised, that there is a “complex immunological interaction and information exchange between mother and foetus, resulting in maternal immunological awareness of the foetus” (Dierselhuis et al., 2012) (Szekeres-Bartho, 2002). The presence of bidirectional transplacental cells and antigens trafficking has been confirmed by the detection of male microchimerism in maternal UCB samples, pregnant with male foetus (Dierselhuis et al., 2012) and specific tolerance toward noninherited maternal alloantigens (van Rood et al., 2009). In 2003, *Marchant et al* reported the presence of mature cytolytic CD8+ T-lymphocytes in newborns with congenital CMV infection, suggesting that intrauterine antigenic stimulation has the potential to produce protective immunity in the foetus, which persists into newborn period (Marchant et al., 2003).

Serological evidence of EBV infection can be found in approximately 97% of pregnant women, however acute EBV infection during pregnancy is very uncommon and can be identified in only approximately 3% of women during pregnancy (Haeri, Baker, & Boggess, 2010), (Haut et al., 2001). Following

Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

primary infection EBV can persist in latent state in infected B lymphocytes for the whole life (Naher, Gissmann, Freese, Petzoldt, & Helfrich, 1992).

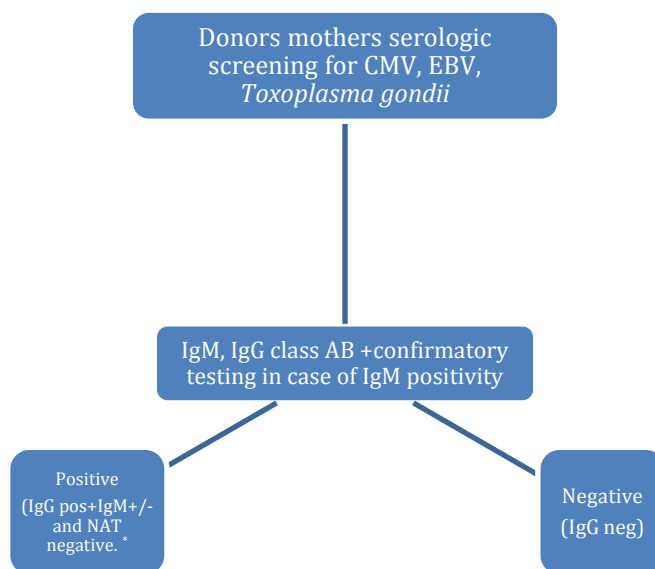
Not much is known about the possibility of congenital transmission of *Toxoplasma gondii* in chronically infected immunocompetent women. However, severely immunocompromised chronically infected women could pass the infection to the foetus, due to the reactivation of latent *Toxoplasma gondii* infection (Kaye, 2011).

I hypothesised, that UCBs collected from the mothers with evidence of previous exposure to the infection (in particular to the CMV and EBV determined by the presence of anti-CMV/EBV IgG class antibodies in peripheral blood) would have had higher total nucleated cell count (particularly in the lymphocyte fraction) reflecting response to the infectious antigen. To prove this theory, I conducted a retrospective study examining the correlation between the cell count of UCBs from donors mothers with/without a history of previous exposure to infections (particularly CMV, Epstein-Barr Virus (EBV) and *Toxoplasma gondii*) (Figure S- 1, Appendix).

4.2. Materials and Methods.

In this study I included UCBs and their maternal blood samples that were processed in two CBBs- Barcelona's and AN CTC, between October 2008 and September 2014. Out of 17,565 UCBs collected during that period, only 4,654 UCBs from AN CTC and 9,754 UCBs from Barcelona's CBB had TNC, CD34+ cell count, lymphocyte and mononuclear cells count documented and therefore were included in the analysis. UCBs laboratory data included for the analysis were: TNC count ($\times 10^7$), CD34+ cells count ($\times 10^5$), lymphocytes (%) and mononuclear cells (%) count. UCBs donors maternal blood samples were tested for the CMV (IgG and –IgM class antibodies (AB) and NAT testing in case of positive IgM AB), EBV (IgG and –IgM class AB and EBNA or other confirmatory test in case of positive anti-IgM), *Toxoplasma gondii* (IgG and –IgM AB and NAT testing in case of positive anti-IgM) (Table S- 1, Appendix). Based on maternal serological findings, UCBs were placed into two groups (with positive or negative serological status separately for CMV, EBV and *Toxoplasma gondii*) and UCB laboratory characteristics were analysed based on this group placement. Maternal positive serological status was defined when IgG class AB was positive with negative or positive IgM class AB (based on manufacturer instructions). Where positive IgM class AB were identified, the blood sample was tested for NAT by polymerase chain reaction (PCR). In the case of a positive PCR result, UCBs were excluded from the analysis. For schematic representation of the study please see

Figure 4-2.



*NAT positive cases were excluded from the analysis.

Figure 4-2 : Study groups: schematic representation

The two CBBs were analysed separately due to the difference in the methods CBU collection/processing (such as timing of cord blood clumping, threshold of TNC and CD34+ cells in CBUs, enabling them for the storage) in order to maintain homogeneity of the group. Barcelonas's CBB practices *in utero* CBU collection and delayed cord blood clumping (which results in 'higher' TNC, as described in previous chapter of my thesis) as opposed to the AN CBB, which do not practice delayed cord blood clumping and *in utero* CBU collection in line with the Royal College of Obstetricians and Gynaecologists guidelines (RCOG, 2009). Also, Barcelona's CBB has a higher threshold for the CBUs TNC (150×10^7) and CD34+ (4×10^6) cells enabling them for the storage. AN has a threshold for the TNC of 140×10^7 and CD34+ of 3.2×10^6 cells, which makes AN and Barcelona's CBBs groups different (personal

Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

communication with Susana G. Gomez, former director of AN CBB and current
Head of Barcelona Transfusion Services).

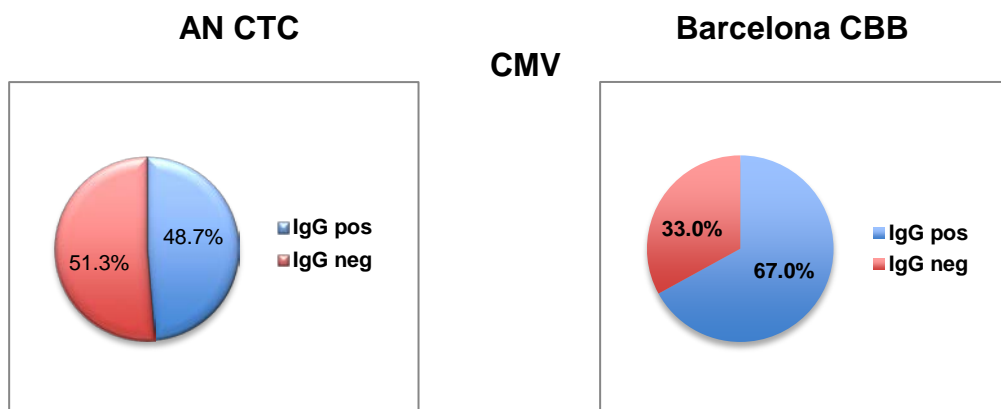
4.2.1. Statistical analysis

Statistical analysis was performed using SPSS software. Descriptive
statistics are shown as arithmetic median and range. To determine
differences between two groups, a non-parametric Mann-Witney U-test
was performed. A value of $p < 0.05$ was considered significant.

4.3. Results

4.3.1. Frequency of positive IgG class antibodies for the CMV, EBV and *Toxoplasma gondii*

Frequency of positive serological status in maternal blood samples from both CBB is shown in Figure VI. Anti-CMV IgG class AB were positive in 48.7% in AN and in 67% in Barcelona's maternal samples. The overall prevalence of positive serologic testing for anti-EBV IgG class AB was more than 95% in maternal samples from both CBBs. Previous exposure to *Toxoplasma gondii* infection as detected by the presence of IgG class AB in maternal samples from Barcelona was 25% in comparison to AN - 10%.



Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

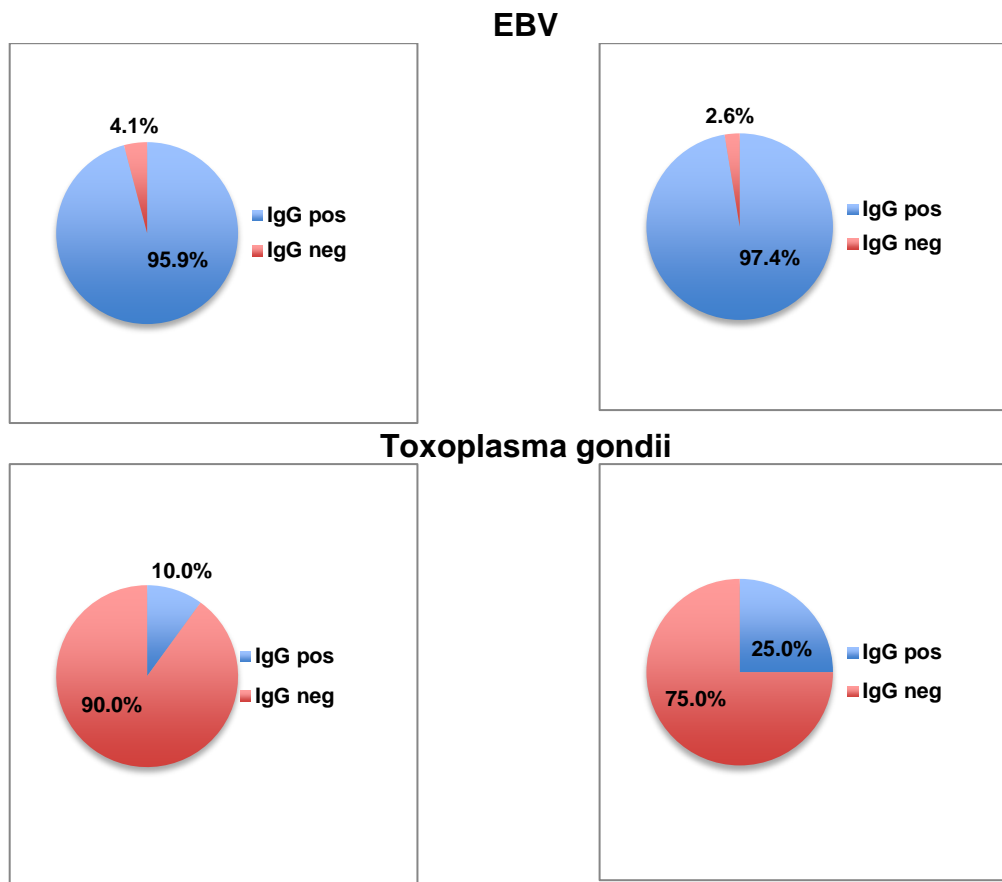


Figure 4-3 : Frequency of maternal positive serologic status for CMV, EBV and *Toxoplasma gondii* in both CBBs

UCBs from donor mothers who tested positive for acute viraemia (by PCR for the CMV and *Toxoplasma gondii* and EBNA negativity in case of the EBV) were not analysed due to the small sample size. Only 30/1,408 (0.2%) of tested maternal samples were positive for the CMV PCR. EBNA negativity was documented in 3 cases and no samples were PCR positive for the *Toxoplasma gondii*.

Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

4.3.2. Cell count in the UCBs from both CBB

The characteristics of the cord blood units (TNC ($\times 10^7$), CD34+ cells ($\times 10^5$), lymphocytes (%) and mononuclear cells (%) count) are summarised in Table 4-1. The cells count numbers parameter expressed in median value. Values shown in parenthesis indicate the range of each value.

Table 4-1 : Summary of UCBs cell count

Result Type	AN CTC	Barcelona
Total N of UCBs	4,654	9,754
TNC ($\times 10^7$)	128.4 (2.2-777.7)	153.3 (\pm 51.7)
CD34+ ($\times 10^5$)	2.93 (0.1-42.2)	6.08 (\pm 3.9)
Lymphocytes (%)	34.7 (1.2-83.2)	36.5 (\pm 10)
Mononuclear cells (%)	49 (4.4-99.1)	40 (\pm 7.3)

Median numbers of TNC in UCBs from Barcelona's CBB were 153.3×10^7 and in AN collected UCBs were 128.4×10^7 . Numbers of mononuclear cells in AN UCBs were 49% and in Barcelona's units were 40%.

4.3.3. Relationship between TNC, CD34+ cells, mononuclear cells and lymphocytes count and maternal CMV serological status.

Each UCB was placed into one of two groups, positive or negative for CMV, as described above.

4.3.3.1. Anthony Nolan Cell Therapy Centre

A univariate analysis was performed to determine the association between maternal CMV status and the TNC, CD34+ cell, lymphocytes and mononuclear cells count. As a result, there is a statistically significant difference in TNC count ($p=0.007$) between UCBs collected from CMV positive mothers (median 130×10^7) when compared to UCBs from CMV negative mothers (median 127×10^7). In addition, a statistically significant difference was found in lymphocyte count ($p=0.003$) between UCBs collected from CMV positive mothers (median 36%) as compared to UCBs from CMV negative mothers (median 35.2%)(Figure 4-4). No difference in the CD34+ cell or mononuclear cell count was found between UCBs collected from CMV positive or CMV negative mothers in AN CTC.

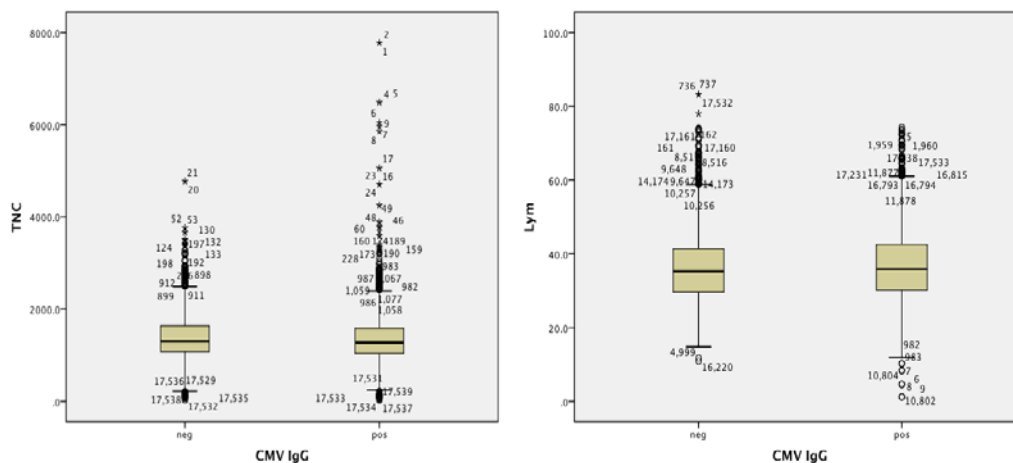


Figure 4-4: Association between maternal anti-CMV IgG positivity and UCBs TNC and lymphocyte count

4.3.3.2. Barcelona's CBB

A univariate analysis was performed to determine the association between maternal CMV status and the total TNC, CD34+ cell, lymphocytes and

Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

mononuclear cells count. As a result, there is a statistically significant difference in TNC count ($p=0.005$) between UCBs collected from CMV positive (median 155×10^7) and CMV negative mothers (median 152.2×10^7). In addition, there is a statistically significant difference in lymphocyte count ($p=0.04$) between UCBs collected from CMV positive (median 37%) and CMV negative mothers (median 36.4%) (Figure 4-5). However, no difference in CD34+ cell and mononuclear cell count was found in UCBs collected from CMV positive or negative mothers in Barcelona's CBB.

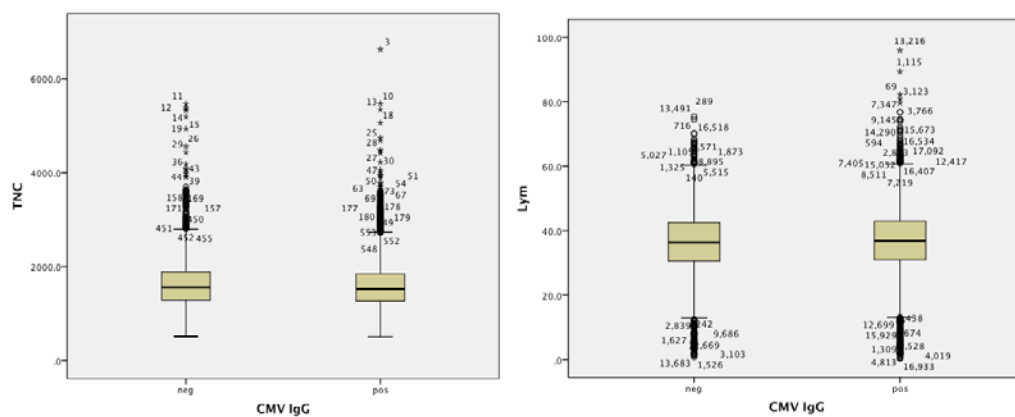


Figure 4-5 : Association between maternal anti-CMV IgG positivity and UCBs TNC and lymphocyte count

4.3.4. Relationship between TNC, CD34+ cells, mononuclear cell and lymphocytes count and maternal EBV serological status.

4.3.4.1. Anthony Nolan Cell Therapy centre

A univariate analysis was performed to determine the association between maternal EBV status and the total TNC, CD34+ cell, lymphocytes and mononuclear cells count. I found no difference in TNC, CD34+ cell, mononuclear cell or lymphocyte count between UCBs collected from EBV positive or negative mothers in AN CTC. Please see Table 4-2.

Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

Table 4-2 : Association between maternal anti-EBV IgG positive AB and UCBs cell content

	TNC	CD34	MNC	Lym
Mann-Whitney U	83775.000	32315.500	73129.500	89108.500
p-value	.331	.591	.688	.991

4.3.4.2. Barcelona's CBB.

A univariate analysis was performed to determine the association between maternal EBV status and the total TNC, CD34+ cell, lymphocytes and mononuclear cells count. A statistically significant difference in lymphocyte count ($p < 0.001$) was found between UCBs collected from EBV positive (median 23%) and EBV negative (median 34%) mothers. In addition, there was a statistically significant difference in mononuclear cell count ($p < 0.001$) between UCBs collected from EBV positive (median 40%) and EBV negative (median 45%) mothers. There was no difference in TNC and CD34+ cell count. Please see Figure 4-6.

Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

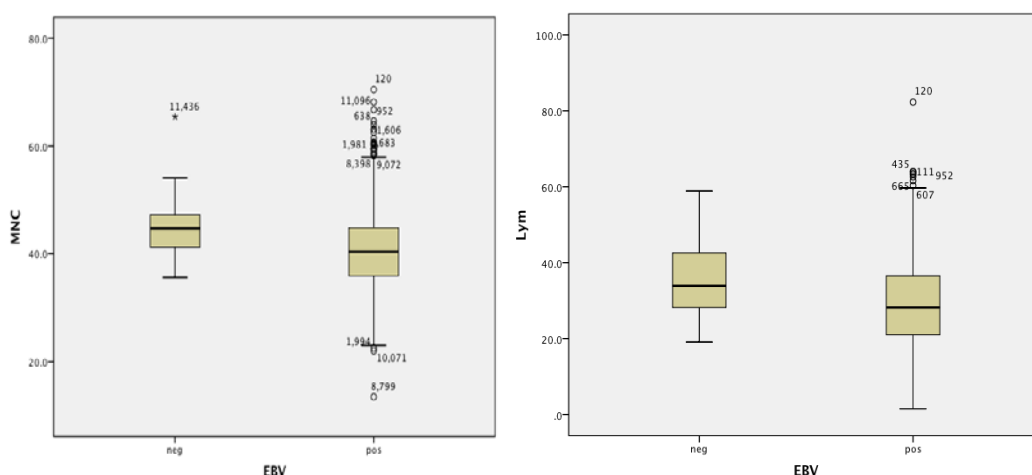


Figure 4-6: Association between maternal anti-EBV IgG positivity and lymphocyte and mononuclear cell count

4.3.5. Relationship between TNC, CD34+ cells, mononuclear cell and lymphocytes count and maternal *Toxoplasma gondii* serological status.

4.3.5.1. Anthony Nolan Cell Therapy centre

A univariate analysis was performed to determine the association between maternal EBV status and the total TNC, CD34+ cell, lymphocytes and mononuclear cells count. The result was no difference in TNC, CD34+ cell, mononuclear cell or lymphocyte count between UCBs collected from EBV positive or negative mothers in AN CTC. Please see *Table 4-3*.

Table 4-3: Association between maternal anti-EBV IgG positive AB and UCBs cell content

	TNC	CD34	MNC	Lym
Mann-Whitney U	83775.000	32315.500	73129.500	89108.500
p-value	.331	.591	.688	.991

Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

4.3.5.2. Barcelona's CBB.

A univariate analysis was performed to determine the association between maternal CMV status and the total TNC, CD34+ cell, lymphocytes and mononuclear cells count. No correlation was found between maternal positive *Toxoplasma gondii* serology and cell count in Barcelona's CBB stored UCBs. Please see Table 4-4.

Table 4-4: Association between maternal anti-Toxoplasma gondii IgG positive AB and UCBs cell content

	TNC	CD34	MNC	Lym
Mann-Whitney U	308443.000	309707.500	289216.000	280282.500
p-value	.795	.897	.580	.055

4.4. Discussion

The specific interest of this study was the relationship between maternal previous exposure to the CMV (as the most common and immunogenic virus) and the donated UCBs cell count. Of the 4,654 mothers evaluated at AN CTC, anti-CMV IgG class AB were present positive in 48.7% cases. Of the 9,754 mothers tested at Barcelona's CBB, 67% showed presence of anti-CMV IgG AB. These results were consistent with previously published data on the prevalence of anti-CMV IgG antibodies. In 19,757 maternal samples available for the evaluation in New York CBB between August 1993 and December 2004, 11,649 (59%) tested positive for the anti-CMV IgG AB (Albano et al., 2006). According to the SaBTO report from March 2012, 50-60% of UK healthy adults showed the presence of anti-CMV IgG antibodies (SaBTO, 2012). In Spain, the prevalence of anti-CMV IgG positivity was reported as 62.8% in between 2013 tested healthy adults (both men and women) in 1993-1994 (Ludwig & Hengel, 2009). The higher frequency of anti-CMV positivity in Barcelona's maternal samples might be explained by older maternal age and difference in socio-economic status between two countries (personal communication with Susana G. Gomez).

In healthy adult individuals, T-cells with predominantly memory compartment play a crucial role maintaining homeostatic balance between the CMV virus and the host (La Rosa & Diamond, 2012). Fetal T-lymphocytes are also capable of responding to CMV antigen as shown in a study on newborn babies with congenital CMV infection maternal CMV infection in pregnancy was

Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

associated with fetal development of mature T-cell responses, including IFN- γ - producing T-cells. This process involved both CD4+ and CD8+ T-cells (Fornara et al., 2011). The same mechanisms might be present in cases, where mother did not have an acute infection during the pregnancy, but had evidence of previous exposure to the virus, as defined by the presence of anti-CMV IgG class AB.

In this study, I found that UCBs, collected from the mothers with evidence of previous exposure to the CMV virus, as detected by presence of the anti-CMV IgG class AB in the blood, had slightly but significantly higher numbers of TNC and lymphocytes. This was observed in UCBs collected and stored in both CBBs. However, this finding should be interpreted with the caution, higher cell count not necessarily only reflects possible exposure to the maternal viral antigens. Many other important factors should be taken into account, such as delivery mode, maternal age, timing of cord blood clamping, gestational age and others, which was scrutinised in my previous chapters. However, it would be of a paramount importance to identify if there is a difference of immunophenotype of the lymphocytes present in UCBs from CMV seronegative or seropositive mothers and performing a tetramer challenge of CBU lymphocytes from CMV seropositive mothers. Due to logistical difficulties, I was not able to perform immunophenotyping, chimerism analysis or functional assays of the cells present in the UCBs from CMV seropositive mothers. However, from the work that has been done on frozen and fresh UCBs at the AN Research institute, it was found that low frequency (<4%) of memory phenotype CD4+ T-cells (CD4+CD45RA-CDRO+) could be identified

Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

in cord blood samples (personal communication, Richard Duggleby, unpublished). In concordance with that finding, in 2014, *Zhang et al* showed the presence of CD4+ T-cells with effector memory phenotype in healthy neonatal blood. “The frequency of those cells was as high as 6% of total neonatal CD4+ T-cells” (Zhang et al., 2014). Moreover, based on X and Y fluorescence in situ hybridisation (FISH) staining, all those cells confirmed to be non-maternal but baby origin. This would suggest that the increase in lymphocytes in UCBs from CMV positive mothers may be due to stimulation of fetal memory T cells from transplacental passage of latent CMV antigens present in the mother.

The presence of such cells in a transplanted UCB unit might result in better clearance of CMV viraemia post transplantation, enhanced alloreactivity, potential GvL effects but possible higher rates of acute GvHD.

As far as EBV is concerned, no correlation between the maternal previous exposure to the EBV and cell count of donated UCBs was found in AN units. However, a positive correlation between anti-EBV IgG class AB presence in maternal blood and mononuclear cells and lymphocyte count was found in Barcelona’s units. This difference can be explained by a bigger sample size of EBV seropositive UCBs collected at Barcelona’s CBB. An increase in the lymphocyte count is a well known effect in response to EBV in healthy adult individuals (Hislop, Taylor, Sauce, & Rickinson, 2007). This study further reaffirms that the fetal immune system is capable of responding to the transplacental passage of foreign antigens in a latent state.

Relationship between donor mothers previous exposure to the viral infections
and UCBs cell count.

I encountered several difficulties in this study; for instance, huge sample sizes and unwieldy spread sheets etc. But the degree of difficulty was alleviated by the highly efficient way AN CTC stores UCB information. Similarly, Barcelona's CBB was extremely helpful and efficient in the way they provided the data. One of the limitations of the study was non-availability of information on immunophenotype of the UCB cells. It is deemed essential that this information is collected and stored.

In conclusion, the findings of the present study show that the number of TNC and lymphocytes is correlated with prior maternal exposure to the CMV, but many other fetal and maternal factors should be considered in multivariate analysis, which was not the part of this current study. With the limitation of current data available it is not possible to determine the phenotype of these cells. Therefore, further studies are needed to address this question of measuring the frequency and specificity of cells in UCBs from CMV seropositive mothers. If we were able to show a clinical significance for the UCBT outcome (in particular enhanced anti-tumour (GvL) effect) when the UCBs are collected from the CMV seropositive mothers, it could change the practice of selecting UCBs for the transplant for patients with haematological malignancies.

Chapter 5. Materials and Methods

5.1. Introduction

This chapter describes the methodology used in the two studies detailed later in this thesis, namely a retrospective study exploring the impact of maternal CMV serological status on UCBT outcome in recipients with acute leukaemia and a retrospective study exploring the impact of maternal previous pregnancies on UCBT outcome in recipients with acute leukaemia.

5.2. Study population

The following eligibility criteria were used to select patients from the Eurocord electronic database: adults and children with primary acute lymphoid leukaemia (ALL), acute myeloid leukaemia (AML) or biphenotypic acute leukaemia, who received an allo UCBT after a 109yeloablative or reduced intensity conditioning regimen in European Group for Blood and Marrow Transplantation (EBMT) centres between 1st January 2000 and 31st December 2012. Recipients who had undergone previous allo HSCT or double-unit UCBT were excluded for the purpose of homogeneity. Patients who received a UCB unit provided from non-European Cord Blood Banks were excluded. A total of 1177 patients were considered for this study (Figure S- 3, Appendix).

5.3. Data collection

Data on patients, transplant procedures and outcome were collected from the electronic Eurocord database (Table S- 4Table S- 5Table S- 6, Appendix). Clinical and outcome data were validated and checked for errors and discrepancies. Data managers and physicians in each EBMT transplant centre were requested to check and complete missing data.

Data on selected unrelated umbilical cord blood units and their maternal characteristics were obtained from European Cord Blood Banks (CBB). All participating CBB received the synopsis of the study and gave their written approval (Figure S- 4, Appendix). Subsequently they were asked to complete the spreadsheet with the required information (Table S- 3, Appendix). Data were checked and validated both by managers at Eurocord and myself. Data managers or clinical staff in each CBB was requested to check and complete missing data. The Institutional Review Board of Eurocord approved the study.

5.4. Study end-points and statistical analysis

The primary end-points were 2-year non-relapse mortality (NRM) and 5-year relapse incidence (RI).

The secondary end-points were 5-year overall survival (OS), 5-year disease-free survival (DFS), neutrophil engraftment at 60 days and incidence of Grade II-IV acute GvHD.

Definitions of outcomes are outlined in Table 5-1.

Table 5-1: Definitions of Outcomes

Outcome	Definition
Neutrophil engraftment	Event time from the date of transplant to first date at which a neutrophil count of $\geq 0.5 \times 10^9/l$ for 3 consecutive days is achieved, without evidence of autologous reconstitution or graft rejection in the first 100 days, with death prior to engraftment considered as competing risk.
Acute GvHD	The time from transplantation to onset of aGvHD or last follow up. Acute GvHD was defined according to the Consensus Conference Classification (Przepiorka et al., 1995)
Chronic GvHD	The time from transplantation to onset of cGvHD or last follow up. Evaluable if surviving without relapse for more than 100 days with sustained donor engraftment. Chronic GvHD was defined according to the Shulman Classification (Vigorito et al., 2009).
Relapse	Defined on the basis of morphological evidence of acute leukaemia in bone marrow, blood or extramedullary organs.
DFS (disease-free survival)	Time from transplant to relapse, death or last follow-up.
NRM (non-relapse mortality)	Non-relapse mortality was defined as death without prior relapse.
OS (overall survival)	Overall survival was calculated from the date of transplant until death or last observation alive.

Median values and ranges were used for continuous variables.

Percentages were used for categorical variables.

Probabilities of neutrophil engraftment, NRM and chronic GvHD were analysed as the cumulative incidence rates and their 95% confidence intervals (Cis), estimating death or relapse as a competing event. Due to the significant amount of missing data, univariate analysis for factors associated with Grade II-IV acute GvHD was conducted using proportions, whilst logistic regression analysis was used for multivariate analysis.

Probabilities of OS and DFS were calculated using the Kaplan-Maier method. The log-rank test was used for univariate comparisons. Multivariate analyses were performed using Cox's proportional hazard regression model for DFS and OS, and Fine and Gray's proportional hazard regression model for other outcomes. Variables that reached a p-value of 0.15 in the univariate analysis were included in the initial models. Variables were eliminated one by one in a stepwise fashion in order to ensure only those which reached a p-value of 0.05 in the final model were included.

The following factors were considered potential predictors of outcome: recipient's age, recipient's CMV status, gender, stage of disease, donor-recipient ABO match, donor-recipient HLA match, graft collected cell dose (TNC and CD34+), year of UCBT (before or after 2008), conditioning regimen (MAC versus RIC), ATG use, maternal CMV status, maternal previous pregnancies, maternal gestational stage and maternal age.

Missing values were found for the important variable of previous maternal pregnancies (N=538, 45.7%). This led me to perform a subsequent subgroup analysis on patients, for whom this variable was available (N=639).

Statistical analyses were performed using IBM SPSS Statistics 20(Copyright IBM Corporation 1989, 2011) and R 2.14.0 (Copyright R Foundation for Statistical Computing, Vienna, Austria, 2011) software packages. For all tests, p-values <0.05 were considered significant.

Chapter 6. Impact of maternal CMV serological status on UCBT outcome in recipients with acute leukaemia

6.1. Introduction

Cytomegalovirus (CMV) status of the graft in the context of allogeneic HSCT has an impact on transplant outcome. Both CMV seropositivity of the recipient and transplantation of seropositive recipient with a seronegative stem cell donor are known risk factors for increased transplant related mortality (TRM) with respect to allogeneic bone marrow or peripheral blood HSCT(Lanino et al., 2008), (Matsumura et al., 2007), (Ljungman et al., 2010).

Delayed T-cell recovery post UCBT has, in particular, been associated with a high rate of viral reactivation (Sauter et al., 2011), (Brown et al., 2010). In UCBT recipients, the data on CMV infection remains controversial. The source of CMV after UCBT is almost exclusively host, because the incidence of a congenital CMV infection is low. Depending on socioeconomic and geographic factors, approximately 0.5-2.0% of neonates are born with congenital CMV infection (Griffiths et al., 2015). Although a UCB may be considered CMV negative at the time of transplant, many UCBs will test positive for CMV IgG due to the transmission of maternal antibodies across the placental barrier (Reynolds, Stagno, Hosty, Tiller, & Alford, 1973). The incidence and risk factors for CMV

reactivation post UCBT have been explored by several studies. In 2006 *Maria S. Albano et al*, assessed the incidence of CMV infection after transplantation of UCB. Post-transplantation CMV infection, was reported in 23% of 1,221 UCB recipients and was associated with recipient's pre-transplantation CMV serological status but not with the CMV serological status of donor- mother's UCB. The group did not analyse the impact of the CBU's serological status on transplant outcome (Albano et al., 2006).

The correlation between CMV infection and disease recurrence in recipients with haematologic malignancies after allogeneic HSCT has been an area of ongoing scientific interest for several years. *Green M et al*, demonstrated a "modest reduction in early relapse risk after adult related and unrelated donor HSCT associated with CMV reactivation in a large cohort of children and adults with haematological diseases" (2,354 study subjects) (Green et al., 2013a). The authors could not identify the exact biological mechanism(s) between CMV reactivation and decreased early relapse observed in allogeneic HSCT recipients. *Cichocki et al.*, had analysed the 1-year relapse rate of 674 paediatric and adult recipients with haematological malignancies after either RIC or MAC conditioning regimen allogeneic related/unrelated HSCT (with CBU as a graft source in 470 cases). In multivariate analyses adjusted for recipient's CMV status, conditioning regimen, donor type (MSD vs. UCB), diagnosis, recipient's gender, disease risk (standard versus high) and prior autologous transplant, CMV reactivation was independently associated with lower relapse risk and increased disease-free survival following RIC, but not following MAC

HSCT. They hypothesised that ‘the lower relapse risk was due to *in vivo* expansion and survival of adaptive NK cells” (Cichocki et al., 2014).

In contrast to the above studies, *M Jeljeli et al*, showed that CMV reactivation post allogeneic HSCT was associated with increased relapse rate in 108 paediatric recipients who received a MAC unrelated/related adult donor or cord blood HSCT for acute leukaemia. Of note is that UCB was the graft source in 17 cases (M Jeljeli et al., 2014).

There is some evidence that the foetus may be exposed to the CMV virus during pregnancy even if the mother shows only IgG antibody positivity and has no symptoms of CMV infection (Paul Griffiths, personal communication). It is hypothesised that this small viral load may lead to an early activation of the fetal immune system and generation of a T- cell immune response but not infection. This immunoreactivity might play a potential role in mediating the graft-versus-leukaemia effect post UCBT. As I found in the study described in Chapter 4, there is a difference in TNC and lymphocyte count between UCBs collected from CMV seronegative and CMV seropositive mothers, with higher number of TNC and lymphocytes in the latter. As discussed in chapter 4, it is possible that these cells represent fetal memory T-cells that developed in response to the CMV antigen present in latent stage in CMV seropositive mothers and that these cells might represent a pool of cells that could mediate GvL effect.

Therefore, the hypothesis of the current study is that maternal previous exposure to viruses (CMV in particular) might enhance antiviral and antitumour

Impact of maternal CMV serological status on UCBT outcome in recipients

with acute leukaemia

properties of the UCB unit with an impact on transplant outcome, such as decreased relapse rate. Therefore a retrospective study which included mother's characteristics of cord blood units delivered by European Cord Blood banks was conducted, the aim being to confirm this theoretical hypothesis.

6.2. Study population

The study population is outlined in section 5.2.

6.3. Materials and methods

The materials and methods of this study are outlined in sections 5.3 and 5.4

6.4. Results

6.4.1. *Patients, disease and graft characteristics*

Patients and transplantation characteristics are summarised in Table 6-1. Median age at UCBT was 13.6 years (range, 0.3-70.7). From a total of 1177 patients, 56.8% (n= 669) were children (i.e. ≤ 18 years). The vast majority of patients (81.5%, n=959) were in clinical remission (CR) and 15.1% (n=178) had advanced disease. At UCBT, 55.6% of patients (n=654) were reported to be CMV seropositive. The median age of UCB was 4.22 years (range, 0.2- 16.75). The median total nucleated cell dose at freezing was $4.74 \times 10^7/\text{kg}$ (range, 0.4 -44.4). The median CD34+ cell dose at freezing was $1.83 \times 10^5/\text{kg}$ (range, 0.6 – 32). Maternal CMV status was reported as positive in 52.1% of cases (n=613) and negative in 43.7% of cases (n=514). In most cases, patients received UCBT with either single (41.1%, n=453) or double (44.9%, n=495) HLA mismatches out of six. Only 117 patients (10.7%) received 6/6 matched UCBs and 37 patients (3.3%) received units with three or more mismatches. A myeloablative conditioning regimen (MAC) was given to 80.5% (n=947) of the patients. The remaining patients (16.7%, n=196) received reduced a RIC. ATG was used in 72.1% (n=849) patients.

Table 6-1: Characteristics of 1177 study patients and UCB grafts

Characteristics	All patients
Recipients (n)	1177
Recipient's age, median (range), years	13.6 (0.3- 70.7)
Recipient's gender	
Male, n (%)	628 (53.4)
Female, n (%)	541 (46.0)
Missing, n (%)	8(0.6)
Diagnosis	
ALL, n (%)	612(52)
AML, n (%)	565(48)
Disease stage at UCBT	
CR, n (%)	959 (81.5)
Other, n (%)	178 (15.1)
Missing, n (%)	40 (3.4)
Recipient's CMV status at UCBT	
Negative, n (%)	430 (36.5)
Positive, n (%)	654 (55.6)
Missing, n (%)	93 (7.9)
TNC dose at freezing, median (range), $\times 10^7/\text{kg}$	4.74 (0.4-44.4)
CD34+ dose at freezing, median (range) $\times 10^5/\text{kg}$	1.83 (0.6-32)
Number of HLA mismatches	
0 -1, n (%)	571 (48.5)
≥ 2 , n (%)	532 (45.2)
Missing, n (%)	74(6.3)

Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

<p>Conditioning regimen</p> <p>MAC, n (%)</p> <p>RIC, n (%)</p> <p>Missing, n (%)</p>	<p>974 (80.5)</p> <p>196 (16.7)</p> <p>34 (2.9)</p>
<p>Serotherapy</p> <p>Yes, n (%)</p> <p>No, n (%)</p> <p>Missing, n (%)</p>	<p>849 (80.1)</p> <p>211 (19.9)</p> <p>117 (9.9)</p>
<p>Maternal CMV status</p> <p>Negative, n (%)</p> <p>Positive, n (%)</p> <p>Missing, n (%)</p>	<p>514 (43.7)</p> <p>613 (52.1)</p> <p>50 (4.2)</p>
<p>Abbreviations: ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; TNC: total nucleated cell; MAC: 121yeloablative conditioning; RIC: reduced intensity conditioning; CMV; cytomegalovirus; UCBT: umbilical cord blood transplantation, CR: clinical remission.</p>	

6.4.2. Neutrophil engraftment

A total of 1025 patients achieved neutrophil engraftment after a median duration of 23 days (range, 4-92). The cumulative incidence of neutrophil engraftment at day 60 was 88.6% (95%CI, 86.8-90.4) (Figure 6-1, A).

In univariate analysis, neutrophil engraftment was strongly associated with several factors Table 6-2.

Table 6-2: Univariate analysis of neutrophil engraftment for 1177 patients with Acute Leukaemia.

Variable	N	% (95%CI)	p-value
Recipient's gender			
Male	620	87.9 (85.4-90.5)	0.05
Female	530	89.6 (87-92.2)	
Recipient's age			
≤ 18 y	662	90.6 (88.4-92.9)	0.4
≥ 18 y	496	85.9 (82.9-89)	
Diagnosis			
ALL	604	88.2 (85.7-90.8)	0.06
AML	554	86.4 (88.4-91.6)	
Disease status at UCBT			
CR	950	90.6 (88.8-92.5)	0.0007
Other	170	78.8 (78.4-85.3)	
Maternal CMV status			
Negative	244	87.7 (84.8-90.6)	0.3
Positive	220	89.8 (87.4-92.2)	
Conditioning			
MAC	378	89.4 (87.5-91.4)	0.8
RIC	86	85.1 (80.1-90.4)	
Median TNC collected x10 ⁷ /kg			
≤ 4.7	224	86.1 (83.3-89.1)	0.03
≥ 4.7	240	91.8 (89.6-94.2)	

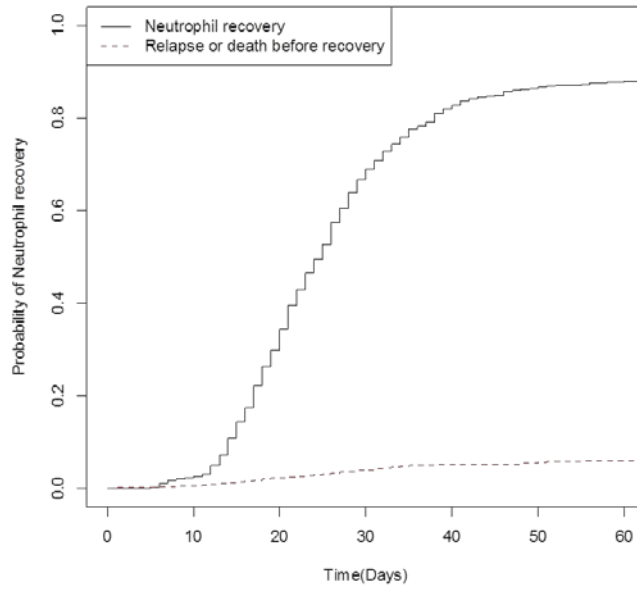
Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

Year of transplantation			
≤ 2008	611	87.2 (84.6-89.9)	<0.0001
≥ 2008	547	90.1 (87.6-92.7)	
Median CD34+ collected x10 ⁵ /kg			
≤ 1.83	236	87.1 (84.3-90.1)	<0.0001
≥ 1.83	228	91.7 (89.4-94.2)	

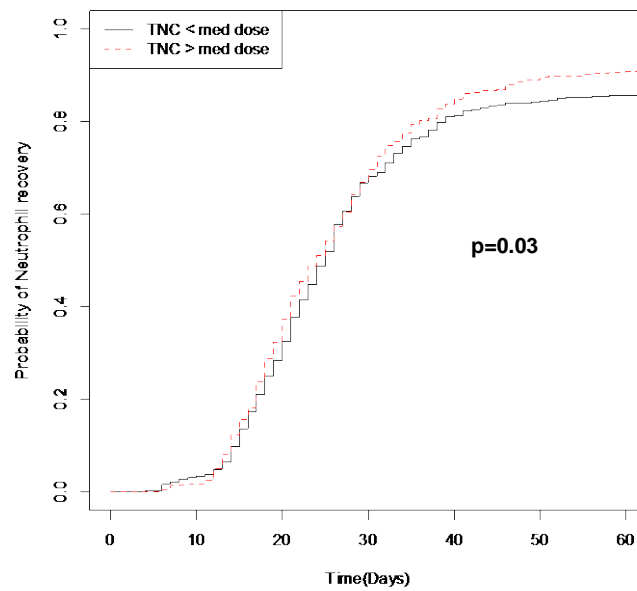
Neutrophil engraftment was strongly associated with collected TNC and CD34+ doses. Those patients who received UCB with a collected TNC below the median for our cohort (i.e. $<4.7 \times 10^7/\text{kg}$), had an engraftment rate of 86.1% (95%CI, 83.3-89.6) compared with an engraftment rate of 91.8% (95%CI, 89.6-94.2) for those patients who received a TNC dose above the median ($p < 0.0001$) (Figure 6-1,B). Patients who received UCBS with a collected CD34+ cell dose below the median for our cohort (i.e. $\leq 1.83 \times 10^5/\text{kg}$), had a rate of engraftment of 87.1% (95%CI, 84.3-90.1) compared with an engraftment rate of 91.7% (95%CI, 89.4-94.2) for those patients who received a CD34+dose above the median ($p < 0.0001$) (Figure 6-1, C). Maternal CMV Serostatus was not associated with neutrophil engraftment.

Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

A



B



C

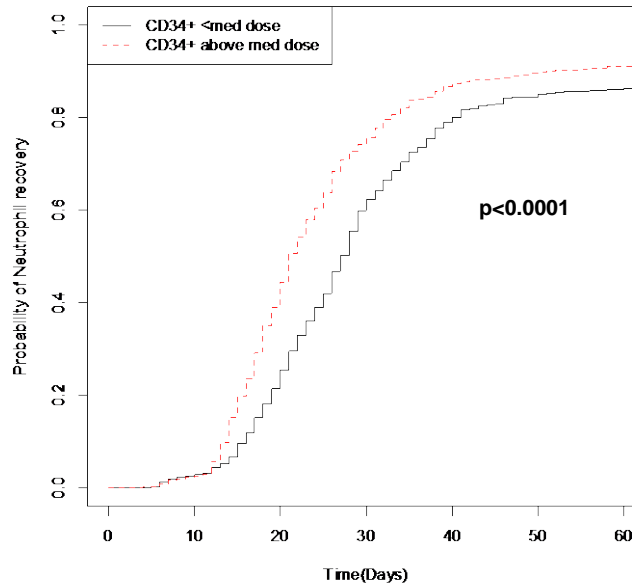


Figure 6-1: Probability of neutrophil engraftment at Day 60. (A): all patients. (B): effect of collected TNC dose. (C): effect of collected CD34+ cell dose.

In multivariate analysis, disease stage (HR=1.3, 95%CI, 1.2-1.5, $p=0.0007$), year of UCBT (HR=1.2, 95%CI, 1.2-1.3, $p<0.0001$), collected TNC dose (i.e. $>$ or <4.7) (HR=1.1, 95%CI, 1.0-1.2, $p=0.03$) and collected CD34+ dose (i.e. $>$ or <1.83) (HR=1.4, 95%CI, 1.3-1.5, $p<0.00001$) were the only factors associated with neutrophil engraftment.

6.4.3. Acute and chronic Graft-versus-Host Disease (GvHD)

Overall 380 (32.3%) patients developed Grade II-IV acute GvHD at a median of 24 days (range, 4-100) after UCBT. Factors associated with Grade II-IV acute GvHD in univariate and multivariate analysis are listed in *Table 6-3*

Impact of maternal CMV serological status on UCBT outcome in recipients

with acute leukaemia

Table 6-3: Factors associated with Grade II-IV acute GvHD in univariate and multivariate analysis.

Variable	Univariate analysis		Multivariate analysis	
	%	p-value	HR (95% CI)	p-value
Recipient's age				
≤ 18 y	38	<0.0001	0.6	0.0001
≥ 18 y	26.2		(0.4-0.8)	
Diagnosis				
AML	30	0.04	n/a	0.6
ALL	35.6			
Conditioning regimen				
MAC	34.3	0.02	0.5	0.05
RIC	25.9		(0.3-0.8)	
Serotherapy				
No	39.8	0.01	0.4	<0.0001
Yes	30.5		(0.3-0.7)	
Median TNC collected X10 ⁷ /kg				
≤ 4.7	25.6	0.01	n/a	0.1
≥ 4.7	32.2			

Of the 785 patients who had engrafted and were still alive at day 100 post-transplant, chronic GvHD (cGvHD) was observed in 188 of cases. The cGvHD was limited in 99 (53%) and extensive in 89 (47%) recipients. The estimated cumulative incidence of cGvHD at one year post –transplant was 20.1% (95%CI, 17.5-23.2).

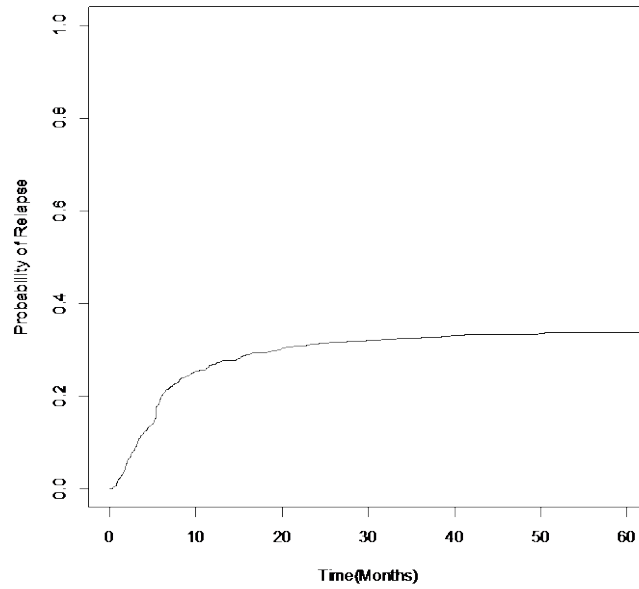
6.4.4. *Relapse*

A total of 352 patients relapsed (of whom 312 died), with a median time to relapse of 8.2 months (range, 1-183). The cumulative incidence of relapse at five years was 33.8% (95% CI, 31 – 36.8) (Figure 6-2, A).

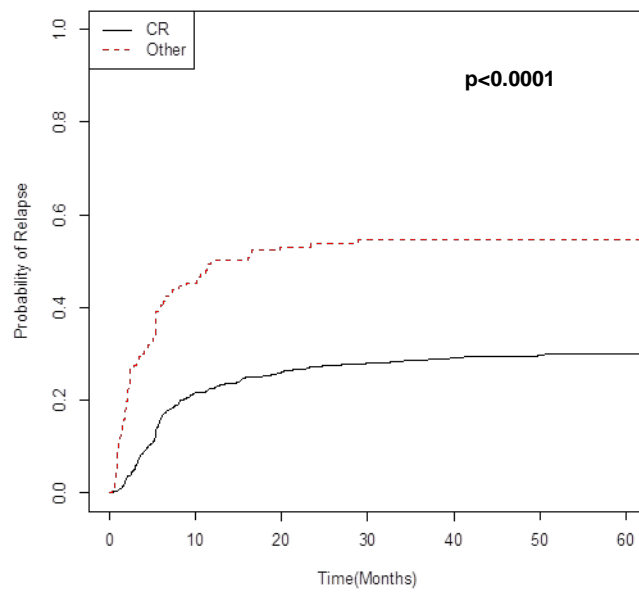
For patients in haematological remission before UCBT, the cumulative incidence of relapse was 30.0% (95% CI, 27 – 33.2) compared with 54.5% (95% CI, 47.1-63.2) ($p<0.0001$) for patients, who presented with advanced stage disease (Figure 6-2, B). In univariate analysis, patients who received MAC, had a cumulative incidence of relapse of 31.9% (95% CI, 28.9-35.3) compared with 42.3% (95% CI, 35.5-50.4) for patients who received RIC ($p=0.02$)(Figure 6-2, C). Patients who received ATG in the conditioning regimen had a cumulative incidence of relapse of 41.8% (95% CI, 35.4 – 49.4) compared to 31.1% (95% CI, 28-34.6) for patients who did not receive ATG ($p=0.009$) (Figure 6-2, D). Maternal CMV status was not associated with relapse ($p=0.79$).

Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

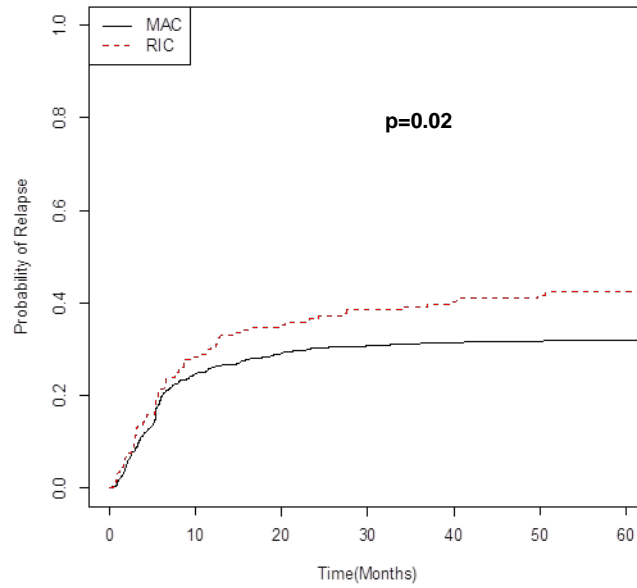
A



B



C



D

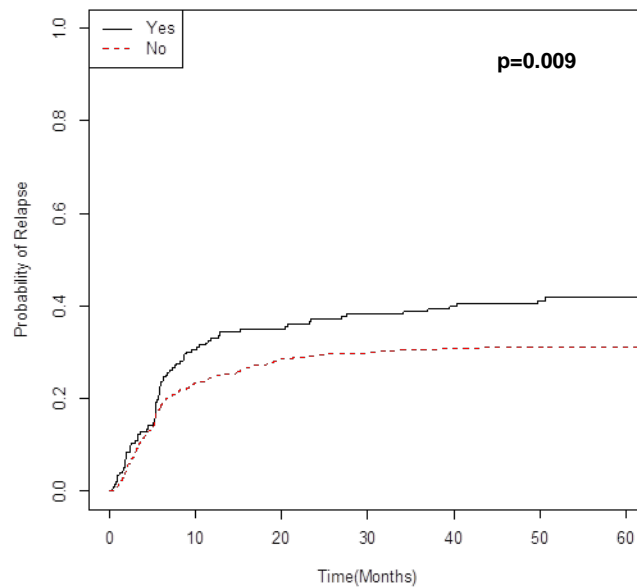


Figure 6-2: Cumulative incidence of 5-y relapse after unrelated UCBT for acute leukaemia. (A): for all patients. (B): effect of disease stage before UCBT. (C): effect of conditioning regimen. (D): effect of serotherapy (ATG).

Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

In multivariate analysis relapse was associated with advanced disease at the time of UCBT (HR = 2.4, 95% CI, 2.1-2.8, $p < 0.0001$), RIC conditioning regimen (HR = 1.3, 95% CI, 1.1-1.5, $p = 0.01$) and ATG use (HR = 1.4, 95% CI, 1.3-1.5, $p = 0.007$), (Table 6-7).

6.4.5. Non-relapse mortality

A total of 312 patients died from non-relapse causes at a median of 105 days after UCBT (range, 1-3626). The 2-year cumulative incidence of NRM after UCBT was 25.4% (95%CI, 22.8-28.4) (Figure 6-3, A).

In univariate analysis, the incidence of NRM was associated with: advanced stage disease ($p < 0.0001$), recipient's age ($p < 0.0001$), recipient's CMV status at time of UCBT ($p < 0.0001$), use of ATG ($p < 0.0001$), HLA mismatches ($p = 0.01$) (Table 6-4).

Table 6-4: Univariate analysis of main factors associated with 5-year NRM

Factor	n	% (95% CI)	p-value
Disease stage			
Advanced	169	39.5 (32.6-47.9)	<0.0001
CR	739	22.2 (19.3-25.4)	
Recipient's CMV status			
Positive	519	29.7 (25.9-34)	<0.0001
Negative	340	16.9 (13.3-21.4)	
Maternal previous pregnancy	320	27.9 (22.8-33.9)	0.03

Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

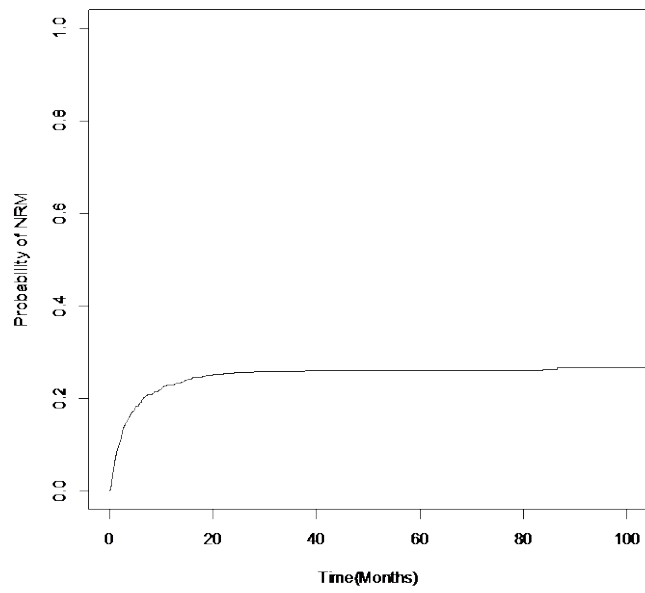
No	319	20.5 (16.0-26.3)	
Yes			
Maternal number of prior pregnancy			
0	320	32.5(27.6-38.2)	0.02
1	218	22.4 (17.4-28.8)	
>1	111	31.3 (23.3-42.1)	
Maternal CMV status			
Negative	411	31.6 (27.7-35.9)	0.5
Positive	487	30.6 (27.3-34.5)	
Conditioning regimen			
MAC	752	30.7 (27.9-33.8)	0.9
RIC	159	30.2 (24.3-37.5)	
Serotherapy			
No	175	22.2 (19.3-25.4)	<0.0001
Yes	841	39.5 (32.6-42.2)	
Number of HLA mismatches			
0-1	457	22.2 (19.3-25.4)	0.01
≥2	421	30.3 (26.2-35.2)	
Recipient's age			
≤18y	537	22.2 (19.3-25.4)	<0.0001
≥18y	407	39.5 (32.4.8-47.9)	

A history of previous maternal pregnancy was associated with NRM. The incidence of 5-y NRM was 27.8% (95%CI,22.8-33.9) for those patients who received UCB from *prima parva* compared with 20.5% (95%CI,16.0-26.3) for patients whose UCB was from a second or greater pregnancy (range, 2-

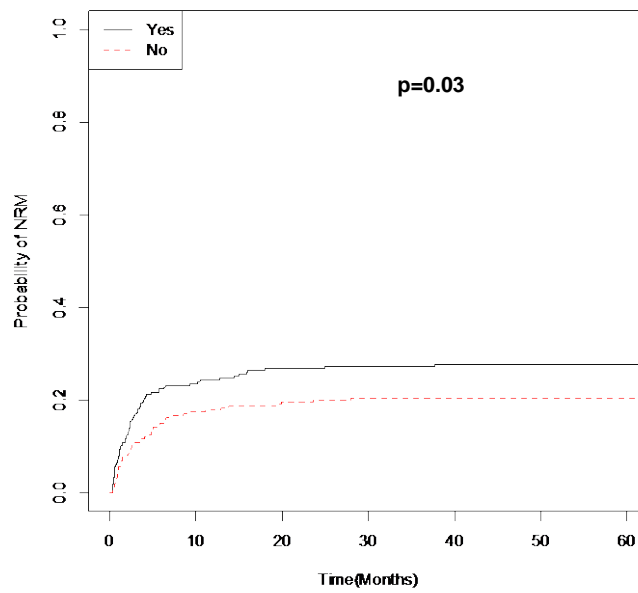
Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

7),(p=0.003),(Figure 6-2, B). Notably, those patients, whose UCB was from the second pregnancy had a lower NRM at 22.4% (95%CI,17.4-28.8) compared with 32% (95%CI,27.6-38.2) for patients, who received UCB from the first pregnancy and 31.3% (95%CI, 23.3-42.1) for patients with UCB from the third or greater pregnancy (range, 3-7), (p=0.02) ,(Figure 6-2, C).

A



B



C

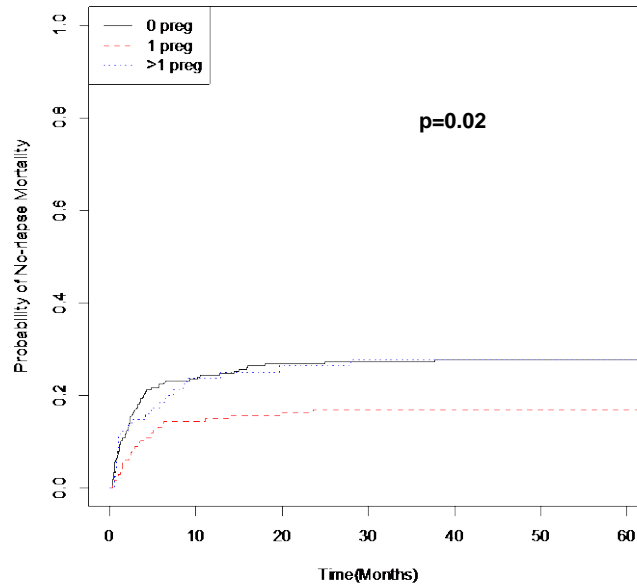


Figure 6-3: Cumulative incidence of 5-y NRM after unrelated UCBT for acute leukaemia. (A): for all patients. (B): effect of maternal previous pregnancies. (C):: effect of number of maternal prior pregnancies.

NRM was not associated with recipient's gender, ABO mismatch, diagnosis (ALL versus AML), year of UCBT (< and > 2008), conditioning (MAC versus RIC) and maternal CMV status (p=0.42).

In multivariate analysis, advanced disease (HR=1.5, 95%CI, 1.3-1.7, p=0.003), recipient's CMV status (HR =1.6, 95%, CI 1.4-1.8, p<0.0001), use of ATG (HR =1.5, 95% CI, 1.3-1.8, p=0.004) remained associated with NRM (Table 6-7).

6.4.6. Overall survival and disease-free free survival.

With a median follow-up of 11.7 months (range, 0.1-183), estimated 5-year probability of overall survival (OS) was 40% (95%CI, 37-43). For patients in CR, 5-year OS was 45.6% (95%CI,42.2-48.8) compared with 10.5% (95%CI,6.7-16.1) for those patients with advanced disease ($p<0.0001$). In univariate analysis, recipient's age ($p<0.0001$), stage of the disease ($p<0.0001$), recipient's CMV status ($p<0.0001$), age of UCB ($p=0.09$), HLA mismatches (0.005), collected TNC dose ($p=0.0001$) and collected CD34+ cell dose ($p=0.02$), history of previous maternal pregnancies ($p=0.007$) were factors associated with OS (Table 6-5), (Figure 6-4).

Table 6-5: Univariate analysis of factors associated with 5-year OS after UCBT for 1177 patients with Acute Leukaemia.

Variable	N	% (95%CI)	p-value
Recipient 's age			
≤ 18 y	664	47.1 (43.2-51)	0.0001
≥ 18 y	508	30.2 (26.1-34.7)	
Recipient's gender			
Male	628	39.7 (35.9-43.7)	0.8
Female	541	40.5 (36.3-44.9)	
Recipient's CMV serology			
Negative	428	46.2 (41.4-51.1)	0.0001
Positive	641	36.9 (33.1-40.9)	
Disease stage at UCBT			
CR	959	45.5 (42.2-48.8)	0.0001

Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

Other	178	10.5 (6.7-16.1)	
Maternal CMV status			
Negative	514	38.3 (34.1-42.7)	0.3
Positive	613	41.5 (37.5-45.7)	
Maternal previous pregnancies			
No	320	36.3 (31-41.9)	0.002
Yes	319	45.7 (40-51)	
Conditioning			
MAC	947	41.2 (37.9-44.6)	0.1
RIC	196	35.2 (28.5-42.5)	
Number of HLA mismatches			
0 or 1	571	44.2 (39.9-48.5)	0.005
≥ 2	532	36.0 (31.8-40.4)	
Median TNC collected x10 ⁷ /kg			
≤ 4.7	559	34.3 (30.3-38.5)	0.0001
≥ 4.7	558	46.6 (42.3-50.9)	
Age of UCB, years			
>4.2 y	584	37.9 (33.9-42.1)	0.09
<4.2	589	42.3 (38.3-46.5)	
Median CD34+ collected x10 ⁵ /kg			
≤ 1.83	531	36.9 (32.7-41.3)	0.02
≥ 1.83	527	43.7 (39.3-48.2)	

Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

UCB storage duration did not have a significant impact on OS, but there was a trend towards worse survival rate after UCBT with older units (i.e. above median storage duration of 4.2 years) ($p=0.09$).

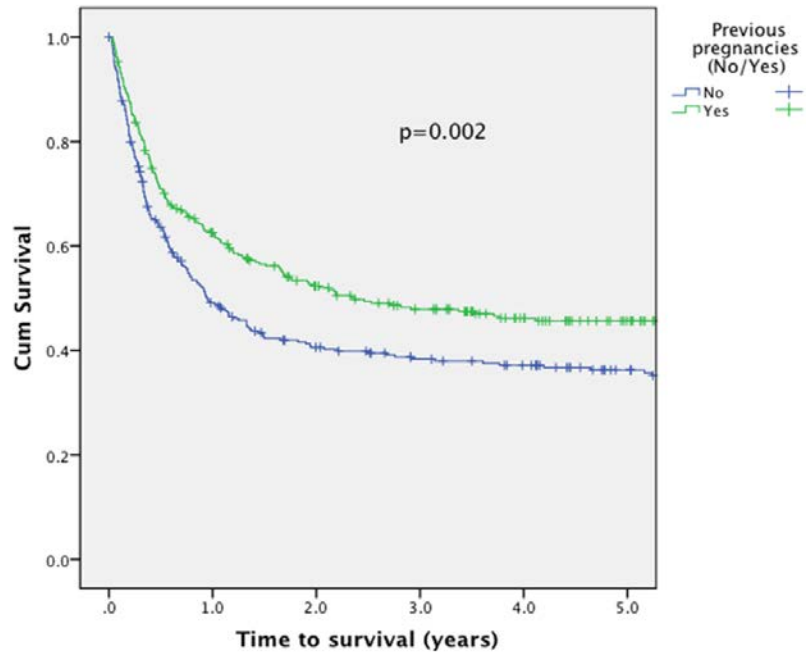


Figure 6-4. Influence of maternal numbers of previous pregnancies to 5-year OS.

Notably, there was no difference in 5-y OS between patients who received UCB from CMV seropositive mothers 41 % (95% CI, 37-45.2) and those who received UCB from CMV seronegative mothers 38% (95%CI, 33.8-42.4), (HR=1.08, 95%CI 0.9-1.2, $p=0.31$), (Figure 6-5).

Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

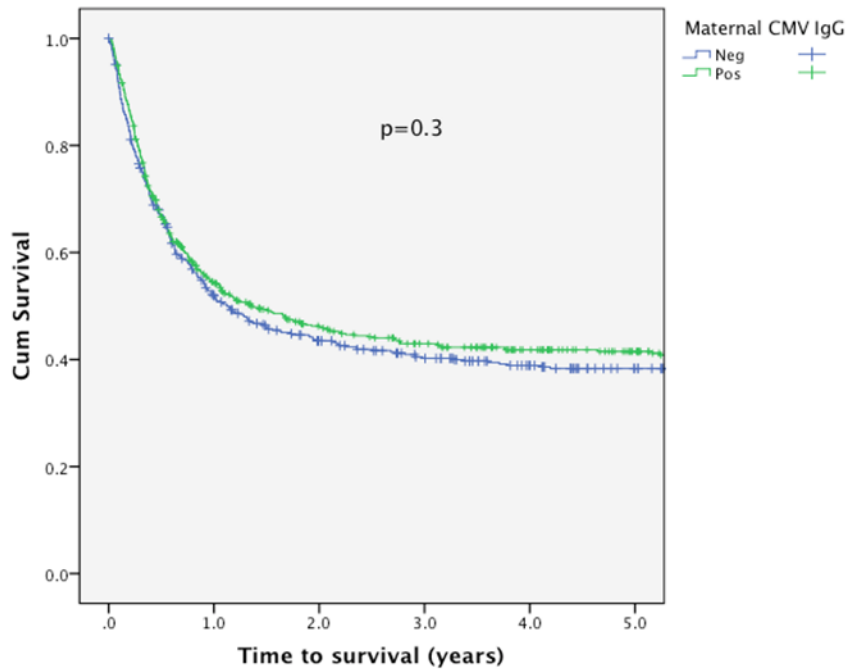


Figure 6-5: Influence of maternal CMV status on 5-year OS.

In multivariate analysis, recipient's age (HR =1.3, 95%CI, 1.1-1.5, $p=0.002$), disease stage at the time of UCBT (HR=2.6, 95%CI, 2.1-3.2, $p<0.0001$) and recipient's CMV status (HR=1.2, 95%CI, 1.0-1.4, $p=0.02$), history of maternal previous pregnancies (HR=0.4, 95%CI, 0.2-0.7, $p=0.004$) and maternal numbers of previous pregnancies (HR=1.4, 95% CI, 1.0-1.9, $p=0.03$) were factors associated with OS (Table 6-7).

The probability of disease-free survival (DFS) at 5 years was 43% (95%CI, 39.7-46.4) for patients in CR and 12% (95%CI,7.4-18.8) for those with advanced disease ($p=0.001$) (Figure 6-6).

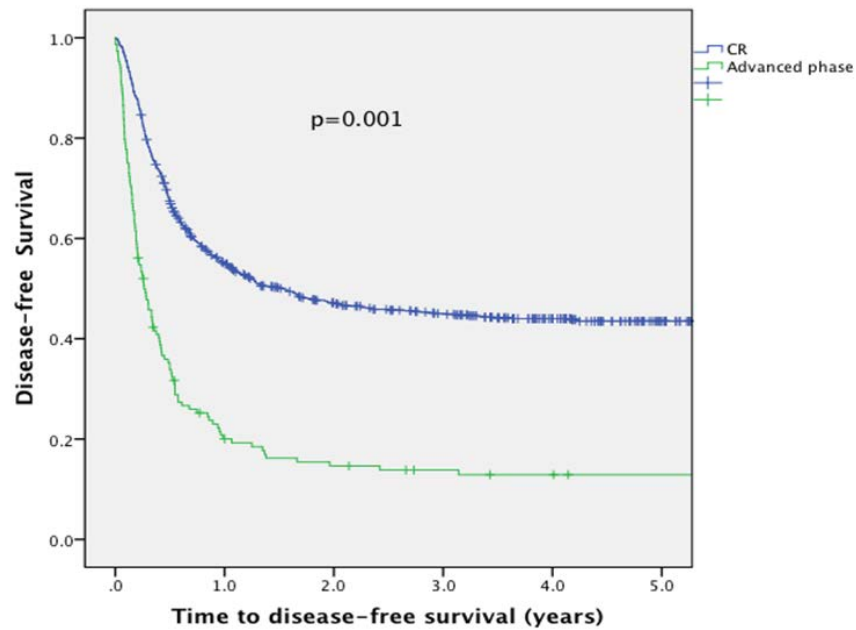


Figure 6-6: The probability of 5-year DFS according to disease stage

Factors, influencing 5-year DFS in univariate analysis are listed in Table 6-6. Notably, history of maternal previous pregnancies ($p=0.009$) and numbers of previous pregnancies ($p=0.004$) are associated with DFS. The 5-year DFS for patients receiving a UCB from a second or greater pregnancy (range, 2-7) was 44.1% (95% CI, 35.5-47.1) compared with 34.4% (95%CI, 29-40.3) if the unit was from a first pregnancy ($p=0.009$) (Figure 6-7). Patients who received UCB from the second pregnancy had superior 5-y DFS at 48.4% (95%CI, 41.4-55.4) compared with patients whose UCB was from the first pregnancy- 34.4% (95%CI, 29-40) or third or greater pregnancy (range, 3-7) – 35.1% (26.1-45.3) ($p=0.004$) (Figure 6-8).

Impact of maternal CMV serological status on UCBT outcome in recipients

with acute leukaemia

Table 6-6: Univariate analyses of 5-year DFS after UCBT for 1177 patients with Acute Leukaemia

Variable	N	% (95%CI)	p-value
Recipient's age			
≤ 18 y	604	46.3 (42.2-50.4)	0.0001
≥ 18 y	452	28.7 (24.6-33.2)	
Recipient's CMV serology			
Negative	399	43.5 (38.5-48.6)	0.005
Positive	598	36.2 (32.4-40.2)	
Disease stage at UCBT			
CR	896	43.5 (40.2-46.9)	0.0001
Other	148	12.9 (8.2-19.6)	
Maternal CMV status			
Negative	455	37.5 (33.1-42.1)	0.3
Positive	556	40.2 (36-44.6)	
Maternal previous pregnancy			
Yes	298	44.1 (38.3-50)	0.009
No	284	34.4 (29.40)	
Maternal number of previous pregnancies			
0	284	34.4 (29-40)	0.004
1	199	48.4 (41.4-55.4)	
>1	95	35.1 (26-45)	
Conditioning			
MAC	947	41.2 (37.9-44.6)	0.1
RIC	196	35.2 (28.5-42.5)	
Serotherapy			
No	198	38.2 (31.6-45.2)	0.9

Impact of maternal CMV serological status on UCBT outcome in recipients

with acute leukaemia

Yes	787	39.5 (36-43.1)	
Number of HLA mismatches			
0 or 1	519	43.1 (38.9-47.4)	0.01
≥ 2	474	34.6 (30.2-39.2)	
Median TNC collected x10 ⁷ /kg			
≤ 4.7	498	33.1 (28.9-37.5)	0.02
≥ 4.7	513	45.2 (40.9-49.5)	
Median CD34+ collected x10 ⁵ /kg			
≤ 1.83	473	35.3 (30.9-39.9)	0.07
≥ 1.83	485	42.4 (38-47)	

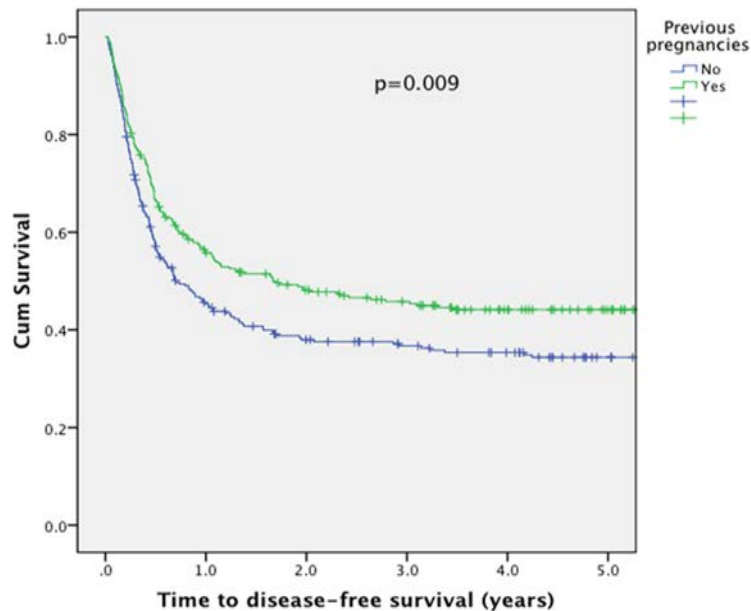


Figure 6-7. Influence of maternal previous pregnancies to 5-year DFS.

Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

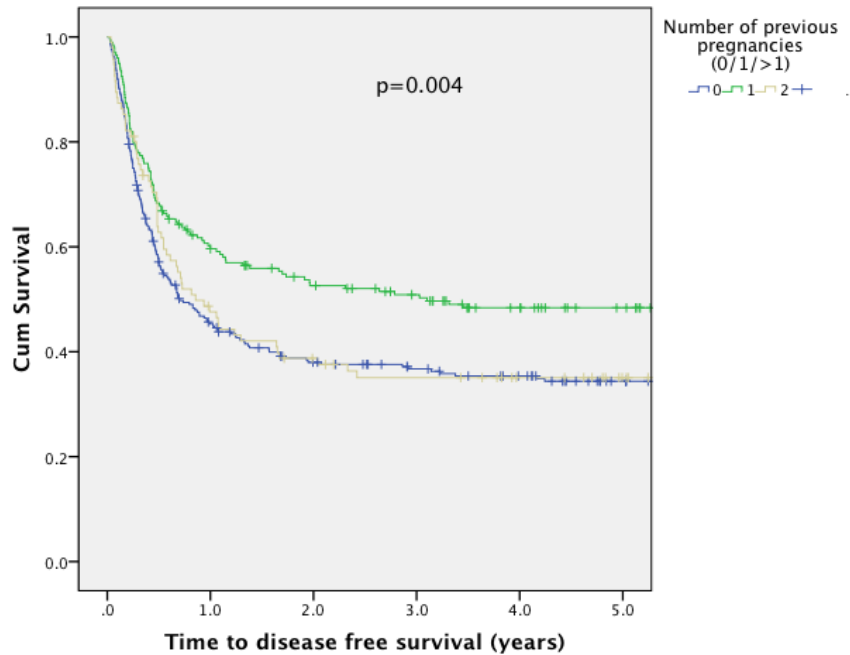


Figure 6-8: Influence of maternal number of previous pregnancies to 5-year DFS.

In multivariate analysis, advanced disease (HR=2.5, 95% C, 2.0-3.1, $p<0.0001$), recipient's older age (HR= 1.3, 95% CI, 1.1-1.5, $p=0.01$), history of maternal previous pregnancy (HR=0.4, 95% CI, 0.2-0.8, $p=0.005$) and numbers of maternal previous pregnancies (HR=1.4, 95% CI, 1.0-1.9, $p=0.04$) were factors which had an effect on DFS (Table 6-7).

Table 6-7: Multivariate analysis of risk factors for each main outcome after UCBT for 1177 children and adults with AL.

Outcomes and unfavourable risk factor	Hazard ratio (95% CI)	p-value
Relapse		
Advanced disease	2.4 (2.1-2.8)	<0.0001
Serotherapy	1.4 (1.3-2.5)	0.007
RIC conditioning regimen	1.3 (1.1-1.5)	0.01
Overall survival		
Advanced disease	2.6 (2.1-3.2)	<0.0001
Older recipient's age (≥ 18 y)	1.3 (1.1-1.5)	0.002
Recipient's CMV seropositivity	1.2 (1.0-1.4)	0.02
Maternal previous pregnancies	0.4 (0.2-0.7)	0.04
Maternal number of previous pregnancies	1.4 (1.0-1.9)	0.03
Non-relapse mortality		
Advanced disease	1.5 (1.3-1.7)	0.003
Recipient's CMV seropositivity	1.6 (1.4-1.8)	<0.0001
Serotherapy	1.5 (1.3-1.8)	0.004

Impact of maternal CMV serological status on UCBT outcome in recipients

with acute leukaemia

<i>Disease-free survival</i>		
Advanced disease	2.5 (2.0-3.1)	<0.0001
Older recipient's age (≥ 18 y)	1.3 (1.1-1.5)	0.01
Maternal previous pregnancies	0.4 (0.2-0.8)	0.005
Maternal number of previous pregnancies	1.4 (1.0-1.9)	0.04

6.5. Discussion

Previous studies reported that several UCB characteristics influence UCBT outcomes. Most of these studies have focused on qualitative and quantitative characteristics of cord blood unit. Important factors (such as cell dose and HLA match) are outlined in the first chapter of my thesis. Evidence of bidirectional foeto-maternal exchange of information suggests that UCB contains cells from both the foetus and the mother. Recent studies indicate that foeto-maternal interaction during pregnancy might enhance the alloreactive potential of cord blood units.

This study analysed was the first to analyse the impact of maternal CMV status on UCBT outcome. This analysis pooled together records of unrelated single-unit UCBT in 1177 adults and children with acute leukaemia.

My retrospective study confirms that cord blood represents a source of haematopoietic stem cells that can be successfully used for an unrelated transplant not only in children but also in adults lacking a suitable adult unrelated donor.

In accordance with previous findings neutrophil engraftment was significantly slower when compared with data on adult unrelated donors (M. Eapen et al., 2010), (F Locatelli et al., 1999). However, the overall probability of donor derived neutrophil engraftment at day 100 was 88.6%

(95%CI, 86.8-90.4). Nonetheless, improving the speed of engraftment remains an on-going challenge. This study confirms previous reports that collected TNC and CD34+ cell dose are associated with the probability of neutrophil engraftment. These factors have been recognised as affecting engraftment in a UCBT setting (L Tucunduva et al., 2014), (Gluckman & Rocha, 2006), (Arcese et al., 2006). Therefore, to improve engraftment, the main effort should be on increasing limited cell dose such as optimising UCB collection techniques, targeted storage of optimal units, further exploration of *ex vivo* expansion and broader use of double cord blood unit transplants. In addition, UCBTs performed before 2008 were associated with lower incidence of neutrophil engraftment. However, the absence of standardised laboratory methods in CD34+ enumeration across the centres precludes us from assessing the implication of this factor.

To summarise, the recent improvements in outcomes are likely to reflect a greater understanding of factors influencing CBU transplant outcomes and the development and use of cord blood selection algorithms.

The present study showed low incidences of acute and chronic GvHD, despite the fact that 45% of patients were mismatched with their cord blood donor at two or more HLA loci. This supports the findings of previous studies on GvHD occurrence after UCBT (Rocha et al., 2001), (F Locatelli et al., 1999).

Non-relapse mortality at 2 years was 25.4% (95%CI, 86.6-90.4). It is significantly lower than in previously published reports which predominantly focused on adult patients (Arcese et al., 2006), (Mary Eapen et al., 2007). It might be explained by the fact, that the current study cohort included a significant number of paediatric patients (57%) and patients presented in CR at the time of UCBT (81.5%). These factors are well recognised for being associated with lower NRM. Other previously described negative prognostic factors, such as recipient's CMV seropositivity at the time of UCBT and the use of serotherapy, were confirmed as being significant in the current study (Y. C. Cohen et al., 2011).

The cumulative incidence of relapse at 5 years was 33.8% (95%CI, 31-36.8). As expected, in this study we confirmed that such factors as advanced disease at the time of UCBT, and use of serotherapy and a RIC regimen were significant factors associated with relapse rate (L Tucunduva et al., 2014), (Ballen et al., 2013), (Rocha et al., 2009) (Franco Locatelli, Schrappe, Bernardo, & Rutella, 2012).

Advanced disease at the time of UCBT and recipient's older age were factors that influenced DFS. Patients who were transplanted in advanced disease had unfavourable DFS rates (43%, 95%CI, 39.7-46.4) while DFS was better in patients transplanted in CR (12%, 95%CI, 7.4-18.8). These figures are similar to those reported in previous studies (Mary Eapen et al., 2007), (L Tucunduva et al., 2014) (Arcese et al., 2006). As with the study performed by Arcese *et al*, I did not observe an effect of HLA disparity on

DFS. A possible explanation could be related to the interaction between cell dose and HLA mismatch (i.e. 'larger units' are more 'acceptable' with greater HLA disparity). Indeed, in our cohort the median collected TNC dose was $4.47 \times 10^7/\text{kg}$. It has been published that an increase in TNC above the minimum threshold of $3.0 \times 10^7/\text{kg}$ is not associated with a reduction in NRM and DFS (V. Rocha, E. Gluckman, 2009).

The use of the RIC regimen in the context of unrelated UCBT for acute leukaemia was addressed by several retrospective studies (Veronika Bachanova, Verneris, Defor, Brunstein, & Weisdorf, 2009) and (Ringdén et al., 2009). In this study, a RIC regimen was associated with increased relapse rate, which was also observed in previous studies on adult recipients with ALL (L Tucunduva et al., 2014), (Ringdén et al., 2009). It was not associated, however, with inferior OS or superior NRM. In contrast to our study, a report from the EBMT group on a series of 449 adult patients transplanted in CR1 for acute leukaemia, showed that NRM was lower after RIC (Ringdén et al., 2009). The difference in results might be explained by the fact that our cohort had a lower proportion of RIC regimen recipients (16.7%).

The rate of 5-year OS in our study was 40%. In line with previous reports, the current study showed that factors that associated with inferior survival rates were: lower TNC dose, older recipient's age, recipient's CMV seropositivity at the time of UCBT and advanced disease (Y. C. Cohen et al., 2011), (F Locatelli et al., 1999), (Rocha et al., 2010) (Mary Eapen et

al., 2007), (Rubinstein et al., 1998), (Arcese et al., 2006) and (L.Tucunduva et al., 2014). As observed in previous reports, this analysis found no link between recipient's gender, diagnosis and conditioning regimen and OS (Luciana Tucunduva et al., 2015), (Arcese et al., 2006) ,(F Locatelli et al., 1999).

This study for the first time provides evidence that the birth order of donated UCBs had an impact on transplant outcome for recipients with acute leukaemia. In this study, I discovered that patients who received UCBs from the second pregnancy had lower NRM and better DFS which translated to superior OS compared with recipients who received UCBs from either the first pregnancy or the third or greater pregnancy. However, the same difference was not observed in the probability of disease relapse. The maternal previous pregnancy effect on DFS and OS was independent factor to other well-established donor-recipient risk factors, including recipient's age, CMV status, stage of the disease and number of HLA mismatches. The major histocompatibility (MHC) identity of the donor and recipient is not the sole factor determining the immunological reactivity in HSCT. In an unrelated donor setting, even if the MHC antigens of the donor are identical to the recipient's, alloreactive transplant reactions (like GvHD and rejection) may occur due to the difference in minor histocompatibility antigens (MiHAs). Previous studies have provided evidence that GvHD could be caused by a number of MiHAs, including H-Y (Choi et al., 2002), (Yang et al., 2003), (Araki et al., 2010). Immunological targeting of H-Y proteins results in a relatively high incidence of acute GvHD when male recipient receives a graft from a female donor in the context of adult unrelated donor HSCT (Stern

at al., 2006). Therefore, it will be logical to assume that in the context of UCBT, the gender of the previous maternal pregnancy might be an important factor. One of the explanatory factors might be the theory of persistent fetal microchimerism in mothers that has been supported by few studies. In 1996, *Bianchi et al* studied venous blood samples of 40 women (32 currently pregnant women and 8 non-pregnant women, who had prior pregnancy). The group demonstrated that male DNA was detected in peripheral blood CD34+ or CD34+CD38+ cells in four women, pregnant with female foetus and six out of eight non-pregnant women. All previous offspring in these women were male gender and one woman had previous pregnancy 27 years prior to the blood collection. Median timing of blood sample for pregnant women was between three to eight months of pregnancy (Bianchi, Zickwolf, Weil, Sylvester, & DeMaria, 1996). In the context of UCBT, the clinical significance of this microchimerism is controversial. On the one hand, the presence of fetal microchimerism in maternal blood induces feto-maternal tolerance and prevent foetus rejection (Godfrey et al., 2005). On the other hand, it might be a potent alloreactivity inducer. For example, in 2010, *Nielsen et al.* performed a study on 499,731 women to analyse the risk of stillbirth in relation to the gender of prior offspring. The report suggested that “women who had previously delivered a boy had a greater risk of stillbirth in subsequent pregnancies”. This effect was explained by a “possible maternal immune response to male -specific MiHAs (H-Y) initiated during pregnancies with boys which persisted for many years” (Nielsen et al., 2010). In addition, “possible association between fetal microchimerism in maternal blood and autoimmune diseases in women have been described” (Miech, 2010) (Sarkar & Miller, 2004), (Leduc, Aractingi, &

Khosrotehrani, 2009), (Maloney et al., 1999). Therefore, subsequent transport of these alloreactive maternal cells acquired during previous pregnancy into cord blood unit of the subsequent offspring could affect UCBT outcome. Unfortunately, in this study, I did not have information of maternal previous pregnancy gender. Therefore, further studies assessing the effect of maternal previous pregnancy gender to UCB's immunogenic potencies (such as superior graft-versus leukaemia effect) are required.

Several studies have been performed to investigate the influence of IPA and NIMA on transplant outcomes. In 1988 *Class et al* found, that HLA broadly sensitised patients failed to produce antibodies against non-inherited maternal antigens (NIMA), but were fully capable of producing antibodies against inherited paternal antigens (IPA) (Claas, Gijbels, van der Velden-de Munck, & van Rood, 1988). This observation led to the hypothesis that recipient HLA antigens which are mismatched with the cord donor but are matched to the donor's NIMA might be permissible HLA mismatches in the context of stem cell transplantation. Higher survival rates after NIMA-matched UCBT supports the theory of NIMA-induced immune tolerance. In 2009, *J van Rood et al* investigated 1121 recipients with haematological malignancies who received a single unit UCB with either single or double HLA mismatches. Of 1121 cases, 79 recipients had at least one HLA antigen mismatch which was matched to the UCBs NIMA. The study demonstrated that recipients, who were transplanted with NIMA-matched units, had better transplant outcomes (lower TRM, better OS and lower incidence of relapse) than recipients whose UCBs were NIMA-mismatched (van Rood et al., 2009). Another study analysed UCBT outcomes

on 164 UCB-recipient pairs for recipients with haematological malignancies. All of pairs in the study were matched in disease, age and transplant procedures (48 NIMA matched and 116 NIMA mismatched pairs) (Rocha et al., 2012). The group demonstrated, that in NIMA -matched pairs TRM was lower in comparison to NIMA-mismatched pairs ($p=0.05$), resulting in superior OS ($p=0.04$).

Considering NIMA-matches as permissible when faced with the choice of multiple HLA- mismatched UCB, might help to identify mismatched units which can be better tolerated (Powley et al., 2016). In addition, it could increase the chances of finding a 'suitable' donor for a greater number of patients. A recent retrospective study on 4707 NIMA-defined UCBs from the UK-British Bone Marrow Registry (BBMR) showed that the cumulative incidence of finding a suitable graft was tripled in ethnic minority recipients and doubled in European Caucasoid recipients if an HLA mismatched but NIMA matched donor was considered (Powley et al., 2016).

Because of the existence of fetomaternal tolerance, IPAs are more immunogenic than NIMAs. In 2012, J van Rood et al. observed that "a higher birth order of UCB donor was independently associated with a reduced incidence of relapse in patients with haematological malignancies" (Rood et al., 2012). The group explained this as "better control of relapse risk through sensitisation to IPA, or an immune "booster" effect associated with repeated pregnancies". However, the precise mechanisms underlying the NIMA and IPA heterogeneity are yet to be established. Unfortunately, in this study, the

information of maternal and paternal HLA typing was not recorded and therefore not assessed.

Clinical evidence of the effect of maternal parity on the allo-SCT setting was observed in several studies. A retrospective analysis on 11365 recipients with haematological malignancies who received HLA identical sibling donor transplants, observed lower rates of acute and chronic GvHD when the donor was younger than the recipient, but no effect of birth order on relapse incidence or overall survival was found (Dobbelstein et al., 2013). The authors explained this outcome by “transient maternal microchimerism with an immunomodulatory (tolerogenic) effect to the ‘older’ sibling present in younger sibling”. In contrast, *Tamaki et. al* reported that maternal grafts were superior in terms of disease-free survival, relapse incidence and mortality in the haploidentical SCT setting (Tamaki et al., 2001). The group had explained this observation by heterogeneity of the NIMA effect, which can be either tolerogenic or immunogenic, as in the latter case. In line with the previously mentioned study on birth order effect in HLA matched sibling HSCT (Dobbelstein et al., 2013), I failed to find a previous maternal pregnancy effect on incidence of disease relapse. However, as mentioned above, *J van Rood et al* observed that relapse incidence in recipients with haematological malignancies was associated with birth order of selected UCB, with “higher” birth order providing anti-leukaemic effect (Rood et al., 2012). The difference between this study and that of *J van Rood* could be explained by a larger sample size in the latter (639 versus 1030 recipients) and by the effect of ethnic background of the study subjects, which

might result in different genetic prognostic factors. In addition, the current study did not examine IPA of the selected UCB-recipient pairs.

Another potential factor to consider is the duration and timing of fetal and mother exposure to non-self-expressed antigens. In that case, maternal pregnancy and delivery outcome (complicated or uncomplicated, i.e. intrauterine haemorrhage etc.), parity and age should be considered. For example, there is a suggestion that feto-maternal haemorrhage either in pregnancy or during delivery leads to “more active” information exchange between the foetus and the mother (Bianchi et al., 1996). In addition it was observed, that high maternal parity was associated with increased risk of *placenta previa*, which a risk of haemorrhage (Shah, 2010). Therefore, it might be possible that both mother and foetus did not have sufficient exposure to non-self-antigens, in the case of uncomplicated pregnancies or deliveries. I did not have information on either maternal pregnancy (i.e. complicated or uncomplicated) or mode of the delivery.

Another factor is maternal age. For example, young maternal age (i.e. less than 18 years) was described to be associated with pre-term deliveries (Kozuki et al., 2013). It was observed that gestational age was associated with quality of collected UCB. UCBs collected from full-term pregnancy have higher TNC count (Solves et al., 2005), (Shlebak et al., 1998). Higher TNC count in UCB is associated with better OS after UCBT (Ballen et al., 2013). Therefore I hypothesise that maternal “younger” age might be associated with inferior UCBT outcome, due to the increased risk of pre-term deliveries. In this study,

full-term pregnancy was associated with better overall survival in univariate analysis when compared with pre-term pregnancy (47.6% and 39.9% respectively, $p=0.03$). Greater number of maternal previous pregnancy was associated with slightly higher risk of pre-term delivery ($p=0.04$), which supports above mentioned theory of TNC dose and gestational age and in current study resulted in inferior OS and higher NRM for recipient of UCBs from third of greater pregnancy.

To summarise, undoubtedly maternal parity, age and other obstetric factors are an important elements in UCBT outcome. Based on the evidence presented in previous reports and results of this study, UCB grafts from the second pregnancy are superior in terms of higher DFS, lower NRM and better OS post-UCBT. The impact of the exact numbers of previous maternal pregnancy is evident, but the reason for this is still not clear. What has become clear is that a very fine balance exists between T- and B-cells tolerance and reactivity. This balance swings in one direction or another over time and under certain conditions and events. When immunotolerance predominates, it results in better survival and lower incidence of GvHD. When the balance fluctuates towards alloreactivity, the graft-versus leukaemia effect is more noticeable, resulting in a lower incidence of disease relapse and perhaps higher rates of GvHD. Factors that could influence potential immunological consequences of exposure to maternal or fetal antigens might be: timing and duration of exposure, quantity and quality of antigen, maternal age, delivery mode and duration of delivery.

Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

Based on evidence presented in this study, I may conclude that, in addition to UCB's "classical" selection criteria (such as TNC dose and currently used UCB-recipient HLA matching), information of maternal previous pregnancies could be taken into account when selecting UCB for transplantation. I suggest that if otherwise equally matched, a UCB from the second pregnancy may be superior to a UCB from first or third or greater pregnancy in terms of improved survival rate. Larger study which captures maternal parity, mode of the delivery, gender of offspring, and maternal and paternal HLA typing, are required to examine birth order effect on UCB transplant outcome.

This analysis has several potential limitations. As in any retrospective study, additional potential unmeasured parameters may exist, for example, recipient's CMV infection post-transplant, transplant centre practice, other maternal factors (like as age, previous pregnancy, gender of previous pregnancy etc.) and even maternal and paternal HLA typing. Unfortunately, missing variables (numbers of pregnancies, for example) was a major limitation when assessing the impact of various factors on transplant outcome in the larger cohort of patients. Some of the follow-up data was unavailable to the registry and some records had missing data.

To conclude, maternal CMV status had no obvious influence on UCBT outcome in this study. However, other maternal factors should also be considered, such as maternal age, parity, UCB birth order etc. In line with previous studies, having disease in CR at the time of transplant and conditioning regimen (MAC and T-cell replete grafts) had a favourable

impact on the risk of relapse. Further larger studies, capturing multiple maternal factors (age, parity, sex of previous babies, HLA typing) and obstetric factors (mode of the delivery, collection technique) are needed in order to confirm findings of this study, that UCBT with CBU from the 2nd pregnancy provides better outcomes in terms of reduced NRM. And most important, to understand the biological mechanism/s of this phenomenon.

Impact of maternal CMV serological status on UCBT outcome in recipients
with acute leukaemia

Chapter 7. Conclusions

7.1. Summary

This thesis presents investigations of factors influencing the quality parameters of cord blood units and the selection criteria of unrelated cord blood units as a graft source for HSCT. A diverse group of parameters contribute to the final cord unit selection criteria. I have written a review of factors implicated in unrelated cord blood unit provision based on the previous experience of different clinical and research groups performing cord blood transplantation across the world. I have designed and carried out five research studies. In this chapter, the outcomes of these studies are summarised. I will also briefly discuss some of the challenges I faced in this work and propose future work that will further contribute to this field.

Chapter 1 summarised the current evidence and practice of unrelated donor provision in the context of unrelated cord blood unit transplantation. It detailed factors influencing unit choice, taking the recipient's diagnosis, age/weight, cell dose and HLA typing of the cord blood unit into consideration. Further factors that have emerged lately have also been described, such as the possible influence of the cord blood unit's NIMA, IPA and KIR ligand on HSCT outcomes. There are only a few studies that have prospectively examined the effect of maternal factors (such as exposure to infections and previous pregnancies) on

transplant outcome and cord blood unit cell content. To my knowledge, my study was the first in-depth investigation into the influence of maternal viral serological status and previous pregnancies on transplant outcomes for patients with acute leukaemia.

The study I conducted in Chapter 2 demonstrated the effect of delivery mode on cord blood unit cell content. It showed that more “stressful” modes of delivery (i.e. instrumental vaginal and emergency Caesarean section) provide cord blood units, which have a higher total nucleated cell content, compared with other types of delivery. Given priority to those collections from above described “stressful” delivery modes might have a clear impact on the use of Anthony Nolan’s resources at cord blood unit collection sites. Besides, the possibility of the introduction of delayed cord blood clumping into standard UK practice should be reconsidered, given the previous experience of its safety and harmlessness, but possible benefit for the collected CBU qualitative parameters.

Chapter 3 focused more on maternal factors that can influence cord blood unit cell content. It explored the influence of maternal previous exposure to viral infections (CMV, EBV and *Toxoplasma gondii*) on the cell count of the collected cord blood unit. The study was conducted in two sites: Barcelona’s Cord Blood Bank and Anthony Nolan Cell therapy Centre. The study found that cord blood units from mothers who had been previously exposed to the viruses, in particular to CMV, had a greater lymphocyte count. Cell dose still remains one of the most important factors when selecting cord blood units for

transplantation. In the future, therefore, maternal serological status might be an important consideration cord blood unit collection and in selection strategy. However, the question that remains unanswered is that of the phenotype of the cells present in cord blood units collected from CMV positive mothers.

Chapter 4 explored cord blood unit-recipient HLA matching at high resolution. Analysis was performed on units, which were selected for transplant with UK patients. This study revealed a high degrees of HLA mismatch between selected donor and recipient when applying matching at high resolution for HLA Class I and Class II genes (-A, -B,-C and –DRB1) opposed to “classical” matching, i.e. low/intermediate level for HLA Class I genes (-A and –B) and high resolution for HLA Class II genes (mainly -DRB1). As previously published, better matching between cord blood unit and recipient at high resolution leads to better transplant outcomes. My study suggests that HLA matching at high resolution level should be implemented when selecting cord blood units. Therefore, it is highly desirable and clinically beneficial for cord blood banks to provide high resolution typing results for all cord blood units at the time of the search.

Finally, in Chapters 5, 6 and 7, I presented a study of the influence of maternal CMV serological status and previous pregnancies on the outcome of unrelated donor single-unit umbilical cord blood transplant for paediatric and adult patients with acute leukaemia. This was the first study to examine the influence of maternal CMV serological status on transplant outcome. I found, that maternal CMV status does not impact on main transplant outcomes. An important finding, however, was that acute leukaemia patients, who received

cord blood unit that was collected from the 2nd pregnancy, had reduced non-relapse mortality and superior overall survival. This knowledge could affect future recording of the data of cord blood unit's quality parameters and could affect UCB selection strategies in the context of UCBT.

7.2. Challenges

Inevitably there were many challenges presented by these projects.

The greatest challenge I encountered during the studies described in this thesis occurred during data collection, particularly for the Eurocord study. Process involved coalition data from many different information sources delivered through different European Cord Blood Banks and Transplant Centres. Often several different spreadsheets were required to provide the necessary information. This proved far more time consuming than I had anticipated.

I needed to visit the Eurocord office in Paris to set up the study and establish collaboration and approval of the study proposal. Whilst I was able to receive the required information from most of the Cord Blood Banks contacted, many of the data points were still missing, in particular, number of previous pregnancies in respect of donor mothers. The same difficulty was encountered in obtaining the information on Cord Blood Unit transplant procedures which Eurocord receives from transplant centres.

This particular study has highlighted the great diversity in the fashion in which data is managed at different transplant centres. Although Eurocord itself has an excellent data acquisition and management methodology in place, the upstream complications in transplant centres presented several challenges.

Sophisticated and complicated statistical analysis of data was also problematic. I needed to become confident working with SPSS and R code. It was a steep learning curve but I have gained a lot in return.

When collecting the data for the collaborative study between Barcelona's Cord Blood Bank and Anthony Nolan Cell Therapy Centre, the enormous size of the data set and spreadsheets was also a challenge. The data analysis therefore proved to be extremely time consuming and complex.

Above challenges however, made me think out-of-the-box and come up with solutions / workarounds. At a personal level this has been a tremendous learning experience for me.

7.3. Future Projects

7.3.1. Immunophenotyping of cord blood units collected from CMV seropositive mothers

I have discovered that the lymphocyte count of cord blood units collected from CMV seropositive mothers is higher than that collected from CMV seronegative mothers. It is of paramount importance to determine the origin and phenotype of these cells. The question to be answered is whether these cells are maternal cells that have crossed the placenta or *de novo* fetal cells developed in response to low level antigen present in mothers who experienced CMV infection before pregnancy. From the work that has been

done and still on-going on frozen and fresh UCBs at AN Research institute, it was found that low frequency (<4%) of memory phenotype CD4+ T-cells (CD4+CD45RA-CDRO+) could be identified in cord blood samples (personal communication, Richard Duggleby, unpublished). Of even greater importance is to discover the clinical implication of these cells.

7.3.2. A study of the influence of previous donor pregnancies on the outcome of unrelated cord blood transplants for malignant disorders.

Following the observation from the retrospective study I performed in collaboration with Eurocord, that previous pregnancies of the UCB maternal donors may influence survival in UCB transplantation, Anthony Nolan are planning to initiate a new larger study using single cord blood transplants for all haematological and non-haematological disorders. Primary data will be obtained from Barcelona's CBB and Eurocord databases. The study will capture birth order and gender of previous pregnancies. If data can be reproduced using a larger sample, it may dramatically change the future selection of the UCB for the transplant, making it more patient-tailored approach.

7.4. Conclusion

In the early 2000s, the usage of UCBT was predominantly limited to paediatric patients due to the greater difference between cord blood unit cell dose and patient weight in the adult recipients group. Since then, a better understanding of the minimum safe transplantable cell dose has improved quality and characterisation of the UCB inventory and the development of the double unit UCBT has broadened the applicability to the adult population. Since the results of UCBT for both adult and paediatric patients are comparable to the results of the adult unrelated donor transplants, cord blood represents an alternative graft source for those patients, who lack a well matched adult donor.

The major challenge for modern cord blood banking is to demonstrate cost-effectiveness and practicality. Through the studies and projects detailed in this thesis, I have been able to identify areas for improvement and potential cost reduction for cord blood banking. It focuses first of all on the cord blood collection sites, given that there is an indication that cord blood units collected via instrumental vaginal deliveries and emergency Caesarean section are more likely to be stored for further clinical use. It continues with maternal characteristics such as history of previous exposure to CMV infection, which leads to a higher cell count in the unit. Studying cord blood unit birth order appears which appears to be significant. Finally, the importance of adopting donor-recipient matching at high resolution level in order to improve cord blood unit transplant outcome.

The adult unrelated donor characteristics, which make it the “perfect match”, are well established: younger age, CMV status, male gender for example. Regarding UCB selection, the “classical “selection strategy has been based on cell dose and HLA matching. But with knowledge of UCB-recipient tri-dimensional relationships, new factors have to be taken into account, such as NIMA, IPA, birth order and gender of previous pregnancies.

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Appendix – Supplemental Tables and Figures

Figure S- 1: Study Proposal of Examining the Association between Maternal CMV Serostatus and UCB Cell Count

Possible Influence Of Cytomegalovirus Status Of The Cord Blood Donor Infant's Mother And Infant's Cord Blood Unit (CBU) On Quality Parameters Of The CBU (ie Total Nucleated Cell Dose (TNC), Haematopoietic Stem Cell Progenitors (or CD34+ cells) Count And Total Lymphocyte Count.

Introduction:

Human cytomegalovirus (CMV) infects most individuals in the world, usually without producing overt symptoms.

Positivity of anti-CMV IgG antibody (AB) in the mother reflects the mother's lifetime exposure to CMV. Anti-CMV IgM AB is a marker of a recent active CMV infection. At the time of the infant's birth, anti-CMV IgG AB were found in 59% and anti-CMV IgM AB in 1.6% of the mothers (*M. Albano et al, 2015*).

The possibility of transmaternal cell flow has been described previously (*M. Dieselhuis et al 2015, B. Momaas et al 2015*). There is growing evidence that intrauterine antigenic stimulation has the potential to elicit immune response in fetus. It is possible, that maternal cells, which were "in contact" with antigen (virus), would gain an access to the placenta, where they might produce pro-inflammatory cytokines and promote cells proliferation. It has been described that CMV could be particularly efficient at promoting the activation and differentiation of CD8+ T cells early in life (*A. Marchant et al, 2003*). Other potential targets of CMV in fetus have to be explored further.

In this study, we hypothesise that CBU obtained from CMV positive mothers might have higher cellularity, in terms of nucleated cell count, lymphocyte count and CD34+ cell count, as they come into contact with the maternal antigen through the transmaternal cell flow.

Materials and methods

We will include in the study CBU which were collected in both Nottingham's Cell Therapy Centre and Barcelona's Cord Blood Bank from October 2008 to September 2014. Only CBU which were proved to be suitable for the clinical use (according to the local standards) will be included in the study. Data on CBU quality parameters will include post-processing and pre-thaw: NC count (per ml or per uL), CD34+ cell count ($\times 10^6$), total lymphocyte count (CD45+) $\times 10^3/\text{ul}$, Data on maternal and CBU serological status (anti-CMV IgG and IgM AB) and viral DNA (when indicated) and CBU quality parameters, will be obtained from the databases of the above mentioned centers. We plan to include both infant's weight (kg) and mode of the delivery (normal vaginal, instrumental vaginal, emergency Cesarean and elective Cesarean section. Those data will be collected from the database of both participating centers.

We plan to evaluate subsequent groups (cohorts) :

Group 1: CMV-IgG and CMV-IgM neg

Group 2: CMV-IgG pos and CMV- IgM AB neg

Group 3: CMV-IgG, -IgM pos and CMV viral DNA pos

Group 4: CMV-IgG, -IgM pos and CMV viral DNA neg

Statistics

To determine difference between means in 4 groups, we plan to perform a parametric ANOVA test, with a post Hoc test to compare all groups. The null hypothesis will be rejected at a P-value of <0.05 .

Table S- 1: Example of a Spreadsheet used for the Study Examining the Association between Maternal CMV Serostatus and UCB Cell Count

CBUID	CBB	NC(10 ⁶)/4+	(x10 ⁶)	MNC (%)	Ly (%)	cCMV IgG	cCMV IgM	CMV PCR	mEBV IgG	cEBV IgM	EBV PCR	mCMV IgG	mCMV IgM	CMV PCR	mEBV IgG2	mEBV IgM	mEBNA mToxIG	G mToxIG	mToxIG	PCR	he delivery
1	706.6749	3.142863	47.56	31	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	762.8043	5.592179	63.96	52.3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	pos	n/a	emCS
1	777.5922	3.268305	49.25	30.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	784.4483	4.100711	60.34	47.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	emCS
1	803.108	3.885562	63.30	49.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	808.1886	4.170156	61.08	47.4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	emCS
1	809.9141	5.228343	52.56	41.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	822.3722	3.15258	67.67	51.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	827.8493	3.161263	71.16	61.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	emCS
1	828.6828	4.379252	57.02	41.6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	emCS
1	833.2009	3.948217	73.27	67.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	emCS
1	833.756	5.260717	56.34	41.3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	833.8062	3.418856	56.75	43.6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	834.4254	3.372856	68.52	56.3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	emCS
1	859.1848	7.014539	50.04	46.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	pos	n/a	NVD
1	865.5441	5.870177	59.54	54.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	pos	n/a	emCS
1	868.0948	3.678552	72.84	54.6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	872.0728	3.401157	69.39	58.3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	874.4	7.6788	69.43	55.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	877.9024	5.225445	52.64	42.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	882.89	4.94695	71.61	52.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	883.9174	4.713607	46.39	35.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	885.7166	3.634246	68.40	60.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	891.9537	4.973567	68.32	54.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	896.1826	3.772251	45.03	37.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	904.6385	3.620273	44.05	29.9	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	emCS
1	907.1194	3.747991	59.78	43.4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	907.6905	6.156979	62.72	45.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	907.7789	3.737264	63.56	46	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	pos	n/a	emCS
1	909.5687	6.530903	51.49	41.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	911.498	4.101283	76.37	64.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	913.6143	5.152613	44.85	24.1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	915.3467	4.62074	55.03	40.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	927.1543	6.90857	51.54	40.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	928.083	4.074653	74.23	61.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	931.2965	4.428856	66.68	51.6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	933.7206	4.562445	61.36	48.4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	emCS
1	934.9517	3.147843	75.36	64.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	935.3329	6.285491	73.57	53.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	935.7831	3.315843	55.50	44.1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	938.8447	3.85244	50.63	38.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	941.1815	3.09939	49.10	36	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	pos	n/a	emCS
1	941.4455	5.371957	48.68	37.6	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	pos	n/a	NVD
1	945.1784	8.793737	63.72	52.1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	pos	n/a	emCS
1	945.3026	4.294503	80.16	72.4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	947.9052	13.26401	67.28	46	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	949.9322	5.001309	45.86	42.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	951.0448	5.61743	55.77	45.2	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	951.8423	2.91247	49.96	37.3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	952.7977	4.804282	65.02	42.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	953.226	8.332416	42.07	29.9	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	pos	n/a	emCS
1	954.7708	4.1714	56.79	50.3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	955.2419	3.659437	34.13	18.4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	pos	n/a	NVD
1	957.4942	3.544413	44.96	34.3	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	pos	n/a	NVD
1	957.9738	4.48397	48.32	34	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	959.6815	3.203905	69.61	62.7	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	960.0483	3.265625	59.83	47.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	960.0697	3.620532	44.59	27.4	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	965.363	4.216252	47.24	31.1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	966.3706	3.945172	66.52	38.9	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	emCS
1	973.8222	3.506919	68.75	57.9	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	pos	n/a	NVD
1	974.6667	7.06392	43.57	35	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	emCS
1	976.8806	3.418798	48.78	37.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	pos	n/a	NVD
1	977.2057	2.951674	51.67	42.1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	978.8713	6.483204	47.94	35.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	emCS
1	979.2639	4.132958	80.28	73.1	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD
1	980.8531	5.239243	56.79	43.8	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	emCS
1	984.4246	8.113308	52.83	28.5	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	pos	neg	n/a	n/a	neg	n/a	NVD

Figure S- 2: Study Proposal for the Project on Donor Recipient Allele-level HLA Matching of Unrelated UCB

Project name: Level of patient/CBU HLA matching in Cord HSCT

Background: Unrelated cord blood is increasingly used as an alternative hematopoietic stem cell source in patients without suitable adult donor. The degree of HLA mismatch is associated with transplant outcomes, such as engraftment, TRM, GvHD. Traditionally HLA-match requirements have been less stringent (antigenic match for HLA-A, -B and allelic match for HLA-DRB1 alleles) with up to 2 alleles mismatch allowed. Recently, the new data has emerged showing HLA-C mismatch being associated with increased TRM in single CBU transplant recipients. Also allele-level HLA matching on both Class I and Class II loci has been recommended to be considered in both single- and double-unit CBT.

Aim: to establish 8/8 HLA matching, and see whether new matching criteria would change CBU selection

Case group: all UK patients transplanted with unrelated CBU in 2013-14 – single or double CBT. N=144

Method:

Patient and CBU HLA typing will be compared based on:

- Current matching criteria: 6/6 with HLA-A and –B at an antigenic level and –DRB1 at allelic level of resolution;
- New matching criteria (recommendations approved by UK Cord Working Group, but not yet published): 8/8 with HLA-A, B, - C, -DRB1 at allelic level of resolution;
- Clinical outcome (OS, TRM, chimerism, neutrophil/platelets engraftment, GvHD) to be collected through BSBMT
-

Staff involved: Head of Specialist Services and Search Advisor (HLA matching) and Medical Officer (patient outcome)

Expected results:

- Understanding of actual situation on patient/CBU HLA matching
- Understanding of potential effect of introducing new matching criteria on CBU selection and availability
-

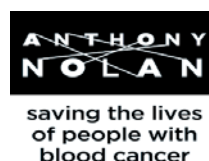
Time frame:

- January 2015 – collecting HLA matching information
- February – March 2015 – collecting patient outcome information
- April 2015 – analysis
- May 2015 – presentation, publication

Table S- 2: Example of a Spreadsheet used for the Study on Donor Recipient Allele-level HLA Matching of Unrelated UCB

Patient	Patient name	Cord ID	UK versus Import	CBU Match grade 6/6	Match grade 8/8 LR	Match grade 8/8 HR	Match grade 8/8, predicted
			Int+Int	4/6	5/8	4/8	NA
			Int+Int	4/6	5/8	4/8	NA
			Import	5/6	NA	NA	6/8
			Import	5/6	7/8	6/8	NA
			Import	5/6	NA	NA	7/8
			Int+Int	4/6	NA	NA	3/8
			Int+Int	4/6	4/8	2/8	NA
			UK+INT	5/6	6/8	NA	6/8
			UK+INT	5/6	NA	NA	5/8
			Import	6/6	8/8	NA	8/8
			Int+Int	5/6	NA	NA	5/8
			Int+Int	5/6	NA	NA	5/8
			UK+UK	5/6	6/8	6/8	NA
			UK+UK	5/6	6/8	6/8	NA
			UK+UK	4/6	6/8	6/8	NA
			UK+UK	4/6	6/8	NA	6/8
			Import	5/6	7/8	6/8	NA
			Import	5/6	7/8	7/8	NA
			Int+Int	4/6	5/8	NA	5/8
			Int+Int	5/6	NA	NA	6/8
			Import	5/6	N/A	N/A	6/8
			Import	6/6	8/8	6/8	NA
			Int+Int	5/6	6/8	6/8	NA
			Int+Int	4/6	5/8	5/8	NA
			Import	5/6	7/8	NA	7/8
			UK	5/6	6/8	6/8	6/8
			UK	6/6	8/8	NA	8/8
			Int+Int	4/6	5/8	5/8	NA
			Int+Int	4/6	5/8	5/8	NA

Figure S- 3: Study Proposal for the Eurocord Study



**Possible Association of Cord Blood Unit and mother's Serological Status
(Cytomegalovirus, Ebstein-Barr Virus and *Toxoplasma gondii*), age, previous pregnancies,
gestational age with Outcomes of Unrelated Donor Single-Unit Umbilical Cord Blood
Transplant (UCBT) for paediatric and adult patients with Acute Leukaemias.**

A study proposal on behalf of Eurocord-CTIWP (EBMT) and Netcord,
And Antony Nolan Research Institute (ANRI)

Principal Investigators:

- Olga Nikolajeva London, ANRI
- Alejandro Madrigal London, ANRI

Co-investigators:

- Vanderson Rocha Eurocord, Hôpital Saint Louis, Paris and London CBB
 NHSBT (Oxford)
- Susana G Gomez Nottingham Cell therapy Centre (ANRI)
- Sergio Querol Barcelona, Cord Blood Bank (Netcord)
- Annalisa Ruggeri Eurocord and CTIWP-EBMT, Hôpital Saint Louis,
 Paris
- Gesine Koegler Düsseldorf, Cord Blood Bank (Netcord)
- Etienne Baudoux Service de thérapie cellulaire et génique, Belgium
- Eliane Gluckman Eurocord, Hôpital Saint Louis, Paris

Statistician

- To be defined

1. Rational

Cytomegalovirus (CMV) status of the graft in the context of allogeneic Haematopoietic Stem Cell Transplant (HSCT) makes an impact on the transplant outcomes. Both CMV seropositivity of recipient and seronegative stem cell donor transplanted to seropositive patient are known risk factors for increased transplant related mortality (TRM) in context of allogeneic bone marrow or peripheral blood HSCT. ⁵

Worldwide, approximately 0.5-2.0% of neonates are born with congenital CMV infection. ⁴ Although we consider cord blood units (CBU) CMV negative at the time of the transplant, many CBU will test positive for CMV IgG due to transmission of maternal antibodies across the placental barrier. ⁸ Group by *Maria S. Albano et al* in 2006, assessed the incidence of CMV infection after transplantation of CBU from unrelated

donors. Post-transplantation CMV infection, reported in 23% of 1,221 CBU recipients, was associated with patient pre-transplantation CMV serology but not with CMV serology in CBU donor's mothers. The group did not analyse the impact of the CBUs serological status on the transplant outcomes.¹

The correlation between the CMV infection and disease recurrence in patients with hematologic malignancies after allogeneic HSCT has been an area of ongoing scientific interest for several years. *Green M et al*, demonstrated a modest reduction in early relapse risk after adult related and unrelated donors HSCT associated with CMV reactivation in a large cohort of children and adults with haematological diseases (2,354 study subjects).² Authors could not identify what was the exact biological mechanism(s) between CMV reactivation and decreased early relapse observed in allogeneic HSCT recipients. In contrast to the above study, *M Jeljeli et al*, showed that CMV reactivation post allogeneic HSCT was associated with increased relapse rate in 108 paediatric patients who received myeloablative (MAC) unrelated/related or cord blood (with CBU as a graft source in 17 cases) HSCT for acute leukaemias. They showed that recovery of anti-CMV and anti-adenovirus immunity of naïve CD4+ T-cells was faster in the non-relapse group.³ The Minnesota group had analysed the 1 year relapse rate of the 674 paediatric and adult patients with haematological malignancies after either reduced intensity (RIC) or MAC conditioning regimen allogeneic related/unrelated HSCT (with CBU as a graft source in 470 cases). In multivariate analyses adjusting for recipient CMV status, conditioning regimen, donor type (MSD vs. UCB), diagnosis, gender, disease risk (standard vs. high) and prior autologous transplant, CMV reactivation was independently associated with lower relapse risk and increased disease free survival following RIC, but not MAC HSCT. They hypothesised that lower relapse risk is due to *in vivo* expansion and survival of adaptive NK cells.¹⁰

In addition, several obstetric, maternal and fetal factors (ie mode of delivery, maternal age and gestational stage) will make an impact on the quality of collected CBU. *Pilar Soves et al*, had investigated the impact of maternal and fetal factors on the cell count of the 300 CBUs. They found that maternal age and gestational stage did not have an impact on the cell dose.⁹ Group of *Omori A*, analysed 916 CBUs and observed significantly higher total nucleated cell count (TNC) in multiparous deliveries. TNC from the first neonates was significantly higher than that from the second, the third and subsequent neonates. TNC from *primiparae* aged 30–34 years was significantly higher than that from the *primiparae* aged 20–24 years.⁶ The group of *Jose Perez et al*, retrospectively investigated the influence of acute CMV exposure on CD34+ cell count in 857 CBUs. CBUs donated by anti-CMV IgM-positive women had lower number of CD34+ cells (2.48×10^6 in anti-CMV IgM-positive donors compared to 1.48×10^6 in unaffected donors).⁷

There are some evidence (Paul Griffiths, personal communication), that foetus may be exposed to CMV virus during pregnancy even if the mother only shows IgG antibodies positivity and have no symptoms of the CMV infection. It is hypothesised that this small viral load may lead to an early activation of the foetus immune system and generation of T- cell immune response but not infection.

Possible association between the maternal serological infectious markers (CMV and possibly others, as *EBV* and *Toxoplasmosis*) and CBU transplant outcomes has to be yet explored.

Therefore, we hypothesise that infant donor's maternal previous exposure to the infections (CMV, EBV and *Toxoplasmosis*), mother's age, previous pregnancies and gestational age might enhance CBUs alloreactive (ie antiviral and antitumor) potentials due to the maternal-fetal cell flow and potential priming of fetal immune cells to viral load and/or other antigens during pregnancy. We assume that maternal previous exposure to the above mentioned antigens might have an impact on the transplant outcomes, such as decreased relapsed rate. Therefore with the aim to confirm this theoretical hypothesis we proposed a retrospective study that includes mothers characteristics of cord blood units delivered by European Cord Blood banks (CBB).

2. Endpoints

2.1 Primary

Non- relapse mortality (NRM) and relapse incidence (RI)

2.2 Secondary

- Engraftment
- Acute and chronic Graft-versus-Host Disease (GvHD)
- Overall Survival (OS)
- Disease free survival (DFS)

3. Materials and methods

3.1 Study subjects

Selection criteria

- 1) Patients (children and adults) with primary Acute leukemias (ALL, AML and biphenotypic) transplanted in EBMT centres from 2000-2012
- 2) First transplant of unrelated single cord blood unit
- 3) European Cord Blood Banks
- 4) At least 2 years follow-up of survivors

3.2 Data collection from the European CBB

European Cord Blood Banks will be contacted to provide the following information:

1. ID number of the selected CBU
2. Date of the collection
3. Selected CBU post-processing, pre-freeze cell count ($CD34^+ \times 10^6$ and $TNC \times 10^8$)
4. Mothers age and numbers of previous pregnancies before the donation (including terminated pregnancies)
5. Gestational age
6. Mothers and selected CBU
 - CMV status (anti -CMV IgG and IgM)
 - EBV status (anti-EBV IgG, IgM and EBNA, or any other confirmatory test in case of IgM positivity)
 - Toxoplasmosis status (IgG and IgM AB)

- Other infections markers if available

3.3 Data from the EUROCORD database

1. Patients and disease characteristics
 - Diagnosis (AML , ALL or biphenotypic) and disease status at transplant (CR1, CR2 or advanced disease)
 - Age at UCBT
 - Weight at the UCBT
 - Gender
 - Year of transplant
 - CMV, EBV status (anti -CMV IgG and IgM, anti-EBV IgG and IgM)
 - Other infectious markers if present
 - 1.
 2. Donor characteristics
 - 3.
 - Level of HLA matching with the patient at 3 loci (For Class I - HLA –A, -B at low/intermediate resolution and for Class II- HLA-DRB1 at high resolution)
 - Infused CBU cell dose (TNC) $\times 10^8$ and CD34+ $\times 10^6$)
 - ABO compatibility

4. Transplantation

- Conditioning regimen (RIC vs MAC)
- Use if serotherapy (ATG or Alemtuzumab)

5. Statistical consideration

5.1 Definitions of outcome

Outcome	Definition
Neutrophil engraftment	First date at which a neutrophil count of $\geq 0.5 \times 10^9/l$ for 3 consecutive days is achieved, without evidence of reconstitution with recipient bone marrow, nor graft rejection in the first 100 days
Platelet engraftment	First date at which an unsupported platelet count of $\geq 20 \times 10^9/l$ for 7 consecutive days is achieved, without evidence of reconstitution with recipient bone marrow, nor graft rejection in the first 100 days
Acute GvHD	According to Seattle criteria.
Chronic GvHD	According to Seattle criteria Evaluable if surviving without relapse for more than 100 days with sustained donor engraftment
Relapse	Defined on the basis of morphological evidence of ALL in bone marrow, blood or extramedullary organs. Haematological, cytogenetic or molecular relapse dates should be reported separately.
TRM	All causes of non-relapse deaths
DFS	Time from transplant to relapse, death or last follow-up

5.2 Statistical methods

The following variables will be considered in a risk factor analysis for outcomes

- Donor characteristics: *CMV, EBV and Toxoplasmosis* serological status of the maternal serum (positive vs negative), maternal age at the time of donation, gestation stage, number of previous pregnancies before the donation
- Patients characteristics (age at UCBT, gender, CMV status (positive vs negative) and other infectious markers if available information is present.

Two separate groups will be analysed.

Group N1: Patients, who received UCBT from anti -CMV IgG and anti- CMV IgM negative CBU.

Group N2: Patients, who received UCBT from anti -CMV IgG positive and anti- CMV IgM negative or positive CBU (NB: CBU with positive anti-CMV IgM are tested for the CMV DNA. In case of the positive CMV DNA, CBU is not suitable for clinical use).

- Disease characteristics: disease status at transplant (complete remission 1 or 2, advanced disease), presence or absence of minimal residual disease at transplantation according to centre definition
- Transplant characteristics: HLA compatibility (based on intermediate/low resolution for Class I – A and B; high resolution for DRB1; ABO compatibility, total nucleated cell dose, CD34+ cell dose, year of transplant, conditioning regimen (RIC vs MAC); serotherapy use).

Cumulative incidence curves will be used in a competing risks setting, death being treated as a competing event to calculate probabilities of relapse, acute and chronic GvHD, neutrophil recovery, TRM. Probabilities of overall and disease-free survival will be calculated using the Kaplan-Meier estimate. The prognostic significance of base-line covariates will be studied by two-sided log-rank tests.

All variables found to have a P value of less than 0.05 will be included in proportional sub-distribution hazard regression model of Fine and Gray (relapse, acute and chronic GvHD, neutrophil recovery, TRM), or as binary covariates in a Cox proportional-hazards model (Disease Free Survival and Overall Survival) with the use of a stepwise procedure and a type I error of 0.05. Relative risks for the association between covariates and events will be estimated with 95 percent confidence intervals.

Statistical analyses will be performed with SPSS (Inc., Chicago) software packages.

6. Publication rules

EBMT rules of publication will be used. All participating centres will receive the draft of the manuscript to allow them to give their input. The order of the authors will be discussed in function of the amount of work given on each manuscript and the number of cases included. All centres will be included either in the authors or if there are too many names in an appendix.

Figure S- 4: Letter sent to CBB giving synopsis of the study and requesting approval

Dear Colleagues,

Eurocord, under the direction of Profs. Eliane Gluckman and Vanderson Rocha, would like to perform a retrospective study titled

“Possible Association of Cord Blood Unit and mother’s Serological Status (*Cytomegalovirus*, *Ebstein-Barr Virus* and *Toxoplasma gondii*), age, previous pregnancies, gestational age with Outcomes of Unrelated Donor Single-Unit Umbilical Cord Blood Transplant (UCBT) for paediatric and adult patients with Acute Leukaemias”

The rationale of this study is to know if there are maternal’ factors (associated with possible alloreactivity) that can impact outcomes of UCBT. We have included only European CBB to facilitate data collection. The Eurocord database shows that 1,326 patients are eligible for this study were transplanted with single-unit cord blood from 2000 through 2012. In order to perform this analysis, we would be very grateful if you could provide the following relevant data on your shipped Cord Blood Units (CBU).

Mother’s CMV serology
Mother’s EBV serology
Mother’s Toxoplasmosis serology
Mother’s date of birth
Number of previous pregnancies to this donation
Gestational age
Date of the CBU collection

Find attached the synopsis for the study and attached an excel spreadsheet/s with a list of CBUs from your bank that met the requirements for this study, containing the information retrieved from the Eurocord database (patients with acute leukemia, who received 1st unrelated single-unit cord blood transplant).

Please indicate whether or not your bank is interested in participating in this study and that you have the above data. If you do not have mothers age, number of previous pregnancies and gestational age.

Please submit the requested information to Eurocord. The deadline for data submission July 1st to the email chantal.kenzey@aphp.fr.

We thank you in advance for your assistance and collaboration, and we are looking forward to hearing from you.

Please, feel free to contact us if you have any questions or concerns regarding this request.

Best regards,

Olga Nikolajeva
On behalf of Eurocord group

Eurocord
Hôpital Saint Louis
1, Av Claude Vellefaux
75475 Paris Cedex 10 France
Phone: +33.1.42.49.48.23
Fax: +33.1.42.46.26.99

Table S- 4: Example of a Spreadsheet on Patients, Graft and Transplant Characteristics and UCBT Outcome Received from the Eurocord Electronic Database – Part 1

IDAA Promise	DOB (recipient's date of birth)	Date of UCBT	CBU ID given by national registry	Date of the CBU collection (same as baby's DOB) dd/mm/yy	Maternal DOB (dd/mm/yy)	Number of previous pregnancies	Gestational age (weeks)	Maternal CMV IgG neg=0 pos=1 ambiguous results =3 not done=7 results missing=9	Maternal CMV IgM neg=0 pos=1 ambiguous results =3 not done=7 results missing=9	Maternal CMV PCR neg=0 pos=1 ambiguous results =3 not done=7 results missing=9	Eurocord database ID	Name of CBB	ID Number of CBB	EBMT Center	EBMT ID	Center	Sex	ABO (A=1, B=2, AB=3, O=4)	Type of acute leukemia	Acute Leukemia type (de novo or secondary)
							42	1	0	7		Programa Concertada Barcelona	5						AML	
							40	0	0	7		Programa Concertada Barcelona	5				female	4	ALL	de novo
						2	0	0	0	7		Besançon	9				male	1	AML	de novo
						2	0	0	0	7		Besançon	9				male	1	AML	de novo
						5	38	0	0	7		Gauting	10				male	4	AML	
						2	0	0	0	7		Besançon	9				female	4	ALL	
						1	1	0	0	7		Besançon	9				female	2	AML	de novo
						1	38	1	0	7		Cliniques Bruc	73				male		ALL	de novo
						1	0	0	0	7		Besançon	9				female	4	ALL	de novo
						1	41	7	7	7		Firenze	51				female		ALL	
						2	39	0	0	7		La Sapienza Ro	54				male		AML	secondary
						1	36	0	7	7		NHS-London	0				male	4	ALL	de novo
						1	1	0	0	7		Bologna	57				female		AML	de novo
						4	1	0	0	7		Besançon	9				female	1	AML	de novo
						0	40	1	7	7		Lügg	70				female		ALL	de novo
						0	39	0	0	7		Málaga	12				female	4	AML	de novo
						0	41	0	0	7		Institut J. Bord	72				female	4	ALL	de novo
						1	0	0	0	7		Besançon	9				male	2	ALL	de novo
						1	1	0	0	7		Bologna	57				male		ALL	
						0	0	0	0	7		Besançon	9				male	4	AML	de novo
						8	0	0	0	7		Besançon	9				female	1	AML	de novo
						4	0	0	0	7		Besançon	9				male	3	ALL	de novo
						0	37	0	0	7		Lügg	70				female		AML	de novo
						1	41	0	0	7		Besançon	9				male	4	ALL	de novo
						1	41	0	0	7		Besançon	9				female	1	AML	de novo
						2	0	0	0	7		Besançon	9				male	2	AML	de novo
						1	1	0	0	7		Besançon	9				female	1	AML	secondary
						2	40	1	0	7		Besançon	9				female	4	ALL	de novo
						2	0	0	0	7		Besançon	9				female		AML	de novo
						0	38.5	0	0	7		Bologna	57				female		AML	de novo
						1	0	0	0	7		Besançon	9				female	1	AML	de novo
						1	0	0	0	7		Besançon	9				male	1	ALL	de novo
						0	0	0	0	7		Besançon	9				male	1	ALL	de novo
						0	0	0	0	7		Besançon	9				male	1	ALL	de novo
						4	1	0	0	7		Besançon	9				male	2	ALL	de novo
						1	0	0	0	7		Besançon	9				female	2	ALL	de novo
						0	1	0	0	7		Besançon	9				male	1	ALL	de novo
						0	40	0	0	7		Lügg	70				male	1	ALL	de novo
						0	40	0	0	7		Cliniques Bruc	73				male	2	ALL	de novo
						1	0	0	0	7		Besançon	9				male	1	ALL	de novo
						1	0	0	0	7		Besançon	9				male	1	AML	de novo
						2	42	0	0	7		Besançon	9				male	4	ALL	de novo
						1	0	0	0	7		Besançon	9				female	1	AML	de novo
						1	1	0	0	7		Besançon	9				male	3	AML	de novo
						0	41	1	0	7		Firenze	51				male	1	ALL	de novo
						0	1	0	0	7		Besançon	9				female	1	AML	de novo
						1	0	0	0	7		Besançon	9				male	3	AML	de novo
						1	1	0	0	7		Besançon	9				male	4	ALL	de novo
						2	1	0	0	7		Besançon	9				male	1	ALL	de novo
						1	0	0	0	7		Besançon	9				male	4	ALL	de novo
						0	1	0	0	7		Bologna	57				female		ALL	de novo
						1	0	0	0	7		Besançon	9				female	1	AML	de novo
						1	40	1	0	7		Málaga	12				female		AML	de novo
						0	0	0	0	7		Besançon	9				female	2	AML	de novo
						1	39	1	0	7		Paoli	55				female	4	ALL	de novo
						4	41	0	0	7		Besançon	9				male	1	ALL	de novo
						1	0	0	0	7		Besançon	9				male	4	ALL	de novo
						1	0	0	0	7		Besançon	9				male	1	ALL	de novo
						1	0	0	0	7		Besançon	9				male	1	ALL	de novo
						1	41.4	0	7	7		NHS-London	0				male	1	AML	de novo
						1	40	1	0	7		Paoli	55				male	4	AML	de novo
						1	0	0	0	7		Besançon	9				male	4	AML	de novo

