CHAPTER 5

5 Principal study: establishing a public umbilical cord blood stem cell bank (UCB SCB) for South Africa

5.1 Introduction

As mentioned in Chapter 4, South Africa faces a large unmet need for bone marrow (BM) transplantation which could be alleviated by establishing a public umbilical cord blood stem cell bank (UCB SCB). A BM registry, under representative of South African demographics, and donor attrition rates of about 25% (Crookes et al., 2007) reduces the possibility of finding an adequate match for haematopoietic stem cell transplantations for many South Africans.

Umbilical cord blood is seen as a viable source to BM (BM itself or mobilised peripheral blood stem cells (PBSCs) for BM transplantation. Establishing a public UCB SCB would therefore be a positive step towards growing South African health care, while similarly addressing the tremendous demand in public health and patient care.

UCB is an important source of haematopoietic stem cells (HSCs). Haematopoiesis, the formation of blood cells, is achieved by lineage-specific differentiation of HSCs. HSCs are undifferentiated precursors of myeloid and lymphoid cells, mainly residing in adult bone marrow, but can also be found in peripheral and umbilical cord blood.

5.1.1 The role of haematopoietic stem cells in bone marrow transplantation

Haematopoietic stem cell transplantations have been used as a successful form of treatment for haematological, non-haematological and certain genetic disorders since the identification of the HLA complex in the early 1960s (Copelan, 2006). Common examples include BM transplantations for treatment of leukaemia, myeloma and aplastic anaemia (Pepper, 2010). Treating an individual with an HLA-matched donor sample greatly reduces the occurrence of graft versus host disease (GvHD) and has an added graft versus leukaemia (GvL) effect (Copelan, 2006).

The success of BM transplantation can be attributed to the innate ability of HSCs and HPCs to self-renew and, subsequently, to differentiate in order to reconstitute the entire haematopoietic system post ablation (Caneth et al., 2010). Haematopoiesis – the formation of blood cells – is achieved by lineage-specific differentiation of HSCs. Numerous studies have been conducted to try to identify the phenotype and characteristics of primitive HSCs, but with limited success. These cells are principally characterised by multipotency (for HPCs) and

pluripotency (HSCs) and the ability to self-renew. It is estimated that approximately 1 in every 100 000 cells in circulating blood is an HSC (Bethesda, 2009). This number can, however, be increased by inducing the release of HSCs from BM into peripheral blood by means of cytokine stimulation (Ivanovic et al, 2009). Chemical signals and regulatory factors (transcription factors, cytokines etc.) involved in the process of HSC differentiation are numerous and their effects are not yet fully understood.

One property of HSCs that enables long-term haematopoietic reconstitution is the phenomenon of 'homing', which in turn, is accompanied by subsequent cellular engraftment. Homing is a controlled process in which circulatory HPCs find their way back to their stem cell 'niches', or sites of origin. These cells display various cellular markers (including CXCR4 – a chemokine co-receptor) that react to chemokine stimuli secreted in the BM stroma, that cause these cells to migrate toward the stimuli and bind to the adhesion molecules in the BM niche (Caneth et al., 2010; Lapidot and Petit, 2002).

HSCs and HPCs in the BM are responsible for replacing dead and dying blood cells and replenishing cells lost in the case of trauma and have the ability to reconstitute the haematopoietic system throughout an individual's lifetime (Wilson et al., 2008). Haematopoiesis thus consists of the self-renewal of primitive HPCs and HSCs, subsequent expansion of the generated lineage-specific progeny and these cells' eventual maturation into unipotent differentiated cells. In order to conserve the pool of pluripotent, self-renewing HSCs and multipotent HPCs, these cells enter a low proliferative state called "quiescence", required to maintain self-renewal capabilities (Tripp et al., 2005). Quiescence is believed to slow down cellular proliferation by keeping the HSCs at rest in the G0 phase and only allowing these cells to enter cellular division at infrequent intervals – generally in response to BM injury or stimulation by, for example, granulocyte-colony-stimulating-factor (G-CSF) (Wilson et al., 2008). These activated HSCs return to their dormant state after re-establishing homeostasis.

Bone marrow transplants can either be allogeneic, from a donor to a different recipient, i.e. another person's cells, or autologous, where the donor is the recipient – i.e. one's own cells (Watt et al., 2007). Autologous transplants are advantageous in posing no risk of rejection or GvHD (although graft failure could result), but could potentially contain intrinsic tumour cells. Autologous transplants also lack the graft versus Leukaemia (GvL) effect seen with allogeneic transplantations (Caneth et al., 2010). The first HLA-matched allogeneic transplant for

treatment of an immunodeficiency took place in 1968 and treatments for aplastic anaemia and leukaemia were routinely performed by the 1970s (Perry et al., 1996; Caneth et al., 2010).

In addition to other problems experienced with BM registries, BM aspirations are painful procedures with the risk of not obtaining adequate numbers of HSCs for successful transplantation (known as a "dry tap"). Currently, most BM transplants are however performed by using peripheral blood (PB) – harvested through apheresis. UCB units on the other hand are readily available and contain HSCs that have high proliferation rates and display a greater deal of immunological tolerance than BM stem cells (Broxmeyer et al., 1990; Fong et al., 2012). UCB units have therefore become a viable alternative source of HSCs for BM transplantation. Since the first successful transplant in 1988, many UCB banks have been established for allogeneic transplantation (Gluckman et al., 1989; Welte et al., 2010). The number of UCB units available for unrelated UCB transplants has increased dramatically over the past ten years, from 129 000 in 2002, to 531 000 units in 2012. Approximately 534 724 UCB units are currently registered with the Bone Marrow Donors Worldwide (BMDW) and the total number of stem cell donors are indicated to be approximately 19.8 million (BMDW; 2011).

5.2 UCB banks around the world

UCB SCs can be cryopreserved for long periods of time. This makes UCB units an attractive source of SCs for BM transplantation, specifically for unrelated donors. In order to benefit from UCB units stored worldwide, UCB banks need to adhere to strict international regulatory standards which assure the quality of the UCB units available to the national and/or international community.

The World Marrow Donor Association (WMDA) in connection with the Worldwide Network for Blood and Marrow Transplantation (WBMT) (amongst others) are working on the development of requirements for standardised practices in cellular therapy. Bodies exist that are necessary national regulatory entities: stem cell registries and stem cell banks.

Stem cell banks are repositories where actual samples are stored. They can be public, commercial, institutional etc. The samples contained in each bank need to be registered at a specific registry where all of the information pertaining to the samples is contained and made available the public (Isasi and Knoppers, 2011). Stem cell registries, on the other hand, do not store specific cell lines. They instead list all information pertaining to the specific stem cell lines

registered with them. Information consists of, for example, the cell line's origin (cell line derivation), where it is stored (which storage facility), and how to obtain that cell line. The information available about the cell lines vary according to the nature of the registry. Registries could be regulatory or more research oriented and this orientation would determine the scope of the registry (i.e. the kind and amount of information available about the samples) (Knowles and Adair, 2007).

The primary goal of the WMDA is thus to ensure the quality and safety of international UCB units by providing minimal operational guidelines to all registries (Hurley et al., 2010). It wants to create unity in practice worldwide, throughout stem cell registries, by unifying them under the umbrella of WMDA standards. For this reason the WMDA facilitates all aspects related to accreditation of bodies involved in cellular therapy using unrelated donor transplants. In addition to compliance with WMDA standards, registries are expected to comply with their own country's governmental regulations and individual transplantation community standards (Hurley et al., 2010). All haematopoietic stem cell registries that would like to become a part of the global registries network would thus be subjected to WMDA accreditation and have to adhere to WMDA standards. These WMDA standards serve as minimal guidelines for registries, but do not cover the requirements of other organisations such as the Joint Accreditation Committee-ISCT (International Society for Cellular Therapy) or the European Group for Blood and Marrow Transplantation (EBMT) (Hurley et al., 2010).

These groups are responsible for overseeing collection/harvest centres, cord blood banking and tissue typing. EuroCord, the international registry for the EBMT, founded its division for international cord blood banking – NetCord – in 1998. NetCord and the Foundation for the Accreditation of Cellular Therapy (FACT) have joined forces in compiling the international standards for UCB collection and banking. FACT was funded by the American Society for Blood and Marrow Transplantation (ASBMT) and the International Society for Cellular Therapy (ISCT) in 1996 (Anon., 2010a).

The respective functions of NetCord and FACT are to oversee the quality of UCB banking and the subsequent clinical use of these UCB units for allogeneic SC transplantation. FACT's mission is to promote quality medical and laboratory practice of cellular therapy through accredited standards, transparent peer review and accreditation.

The Standards are intended to ensure high standard medical and laboratory practices throughout the whole process of UCB banking and storage. This is particularly important in order to be able to reliably and consistently reproduce high-quality UCB products, intended for routine transplantation purposes.

The standards as described by NetCord-FACT entail: "donor management, collection, processing, testing, cryopreservation, storage, listing, search, selection, reservation, release, and distribution to clinical programs". It lists the requirements for UCB collection, sample screening, processing and cryopreservation, storage and characterisation of these units, as well as processes involved to locate specific units intended for release for subsequent administration. Handling of UCB units, including transport or shipment, is also included and detailed in the NetCord-FACT International Standards for Cord Blood Collection, Banking and Release for Administration manual, 4th Edition (Anon., 2010a).

Important additional parameters that need to be standardised are listed by Watt et al., (2007) to be: "(1) transit times and storage temperatures following harvest, (2) pre-processing prior to cryostorage, (3) the selected cryoprotectant, (4) cooling and thawing rates, temperatures, and protocols, and (5) longer-term storage temperatures" (Watt et al., 2007).

It is thus of utmost importance to ensure from the outset that all UCB banks, registries and UCB collection sites adhere to the requirements and standards as stipulated by the WMDA and other international regulatory bodies.

5.2.1 Public vs. private banking

Since UCB units can be cryopreserved for extended periods of time, they serve as vital reserves of UCB units for use in allogeneic transplantation. Many banks have thus been established worldwide, in order to generate an ever-increasing pool of potential HLA-matched UCB units that could be accessed both locally and internationally (Malgieri et al., 2010; Armson, 2005).

5.2.1.1 Public banking

Public banking consists of the anonymous donation and subsequent storage of UCB units for unrelated, allogeneic transplantation (Jordaan et al., 2009). According to the WMDA (2006), more than 1,500 allogeneic transplants occur worldwide each year and are steadily increasing (Anon, 2006). These banks operate out of altruism and mutuality for the purpose of benefiting the public (Jordaan et al., 2009).

Public banks operate on a not-for-profit basis, and any member of the public can donate their UCB blood and access to UCB units is equal for all members of the public, provided that the recipient of a unit is an adequate HLA-match. Many professional organisations and national governments support public banking and its successful application has been extensively documented (Anon, 2006).

A sub-category included under public banking is what the WMDA classifies as "Medically Indicated, Directed Family Cord Blood Storage". In this case, some public banks would provide storage of a UCB unit intended for family usage, where a family with a sickly child wishes to store the UCB of a second expected sibling for the treatment of the first, provided that the first child could potentially benefit from a UCB transplantation (Anon, 2006). There is a 1/4 (25%) chance per sibling of finding an adequate HLA-match between siblings, which thus increases the likelihood of using the specific sample.

5.2.1.2 Private banking

The WMDA defines private banking as banking for autologous or family storage. These banks differ mainly from public banks in that they sell their service of storing UCB units for exclusive use of the donor family and at the discretion of the donor. Unlike public banks where all units are anonimised and the public has equal access to any of these units, people storing their UCB privately retain the right to exclusive access to the unit (i.e. autologous use or use within the family) (Jordaan et al., 2009).

Private banks operate on a for-profit basis, often charging exorbitant fees (usually between $1,000 to $1,500 USD) excluding an annual storage fee (circa $100). They extensively market their services to the public. Their methods often create contention among cellular therapy and transplantation communities since they may hinge on false advertising and incorrect portrayal of the current state of UCB transplantation. Furthermore, patients that store privately often do not have known risk factors that would justify personal usage of the units, with very low likelihoods of these samples ever being used (Jordaan et al., 2009; Anon, 2006).

5.2.2 Controversial aspects contributing to the public-private debate

The current debate around public vs private UCB banking centres mainly around the fact that commercial UCB banking leads to many ethical dilemmas and – many believe – should thus be avoided (Thornley et al., 2009; Sullivan, 2008). The European Union Group on Ethics' stance

against private (otherwise known as "commercial") UCB banking is that it is unethical since private banks sell a service without any immediate tangible use regarding therapeutic options (Malgieri et al., 2010; Anon, 2004).

Proponents for private banking on the other hand maintain that each individual has the freedom of choice to choose where to store his own UCB units and should not be prohibited to do so (Jordaan et al., 2009).

Commercialisation of UCB banking leads to the following main ethical dilemmas: 1) Some private banks having been found to incentivise doctors to recruit patients for private storage; 2) false marketing and advertising where current benefits of UCB storage are overstated; 3) patients signing informed consents without being properly informed of the processes and options involved in UCB banking and 4) some private banks adopting for sales approaches that pressure patients into giving informed consent by playing on the parents' feelings, implying that they are not good parents if they do not store their child's UCB unit for 'biological insurance' (Anon, 2007; Petrini, 2010; Anon, 2006).

5.2.3 Other factors requiring regulatory oversight

5.2.3.1 Regulation and accreditation

Regulations for UCB banks are still being refined and adapted in order to provide for the needs of the public whilst maintaining transplantation excellence, unit safety and donor anonymity. The most recent regulations (although partially still incomplete) for South African SCBs have been published in the March 2012 Government Gazette (Motsoaledi, 2012)). These regulations stipulate the use of SCs, record keeping and reporting on obligations, duties of the health officer, inspection and control measures, traceability, data protection and confidentiality, SC quality and safety, SC quarantine, processing and storage, distribution and SC bank relationship with third parties.

All UCB banks need to adhere to strict regulatory requirements and need to comply with accreditation standards as determined by the WMDA and local government authorities in cellular transplantation (as mentioned previously). At the moment, however, regulations pertaining to cellular transplantation for South Africa are still incomplete (Pepper, 2012).

Running an UCB SCB requires a great deal of financial resources. Public UCB banks are run on a cost-recovery basis and not on a for-profit basis as is the case with private banks. The money to

run public banks often comes from government institutions such as hospitals, medical centres or non-profit organisations (who often don't have adequate funds to maintain these services to the public) (Malgieri et al., 2010; Bellomo, 2006; Anon, 2004).

Private banks, on the other hand, often have shareholders and thus operate on a for-profit basis. They encourage parents to donate their child's UCB unit mostly for autologous use, but with the option of allogeneic use amongst close relatives. This drive to bring in profit and to keep the shareholders happy often leads to questionable marketing and advertising campaigns, inappropriate informed consent procedures, information being accessible to the public, and advertisement / campaigning (Malgieri et al., 2010).

Donor identity, sample anonymity and traceability and patient safety are important factors that require strict regulation (Malgieri et al., 2010). In an effort to bring some form of regulatory oversight into the practice of UCB transplantation, the Human Tissue Authority (HTA) in the UK has put a measure in place to ensure the safety of UCB transplantation in July of 2008. It put into practice the requirement that any UCB bank (public and/or private) must be licensed by them, prior to the release of any UCB unit for transplantation purposes to hospitals in the National Health Services (www.hta.gov.uk).

5.2.3.2 Obtaining informed consent

Information given to the public should be scientifically correct and as extensive as necessary in order for each parent to make a truly informed decision regarding the banking of their child's UCB unit.

There are many aspects involved in obtaining informed consent from a potential UCB donor. Some of the elements, stated by Beauchamp and Childress (2001), include autonomy of the individual giving consent, his or her understanding of the process of UCB donation and banking – both public and private - and his or her voluntary participation in giving consent, to name but a few aspects. It is important that each individual makes an autonomous decision regarding his UCB donation, without being coerced into making a decision or being subjected to biased and false advertising (Petrini, 2010). Obtaining consent from a mother at an appropriate time is also crucial, since it is generally agreed that obtaining informed consent from a mother in labour is a questionable practice, raising many ethical concerns (Petrini and Farisco, 2011).

Aspects that need to be discussed during the informed consent process are set out in the paragraphs below.

5.2.3.3 Informing patients of allogeneic or autologous use of UCB units

Often, patients are misinformed regarding current and potential future therapeutic applications of UCB units (Petrini, 2010). According to research done by Fox et al. (2007), many patients – and especially those planning to store their UCB units privately – had an insufficient understanding of the processes and options involved in UCB banking.

Few people in the general public are educated in current and potential future applications of UCB. Often, private banks promise cures for diseases for which no clinical results have been generated, such as the use of UCB to cure Parkinsons, ALS, MS, diabetes etc. (Anon, 2006). Many are thus misled into believing that these stored UCB units are a form of 'biological insurance' to treat some of the abovementioned disorders (Anon, 2007).

5.2.3.4 Likelihood of requiring UCB units for autologous transplantation

Private banks have frequently been found to neglect to tell the clients of the minimal likelihood of using one's own UCB unit. Primarily two reasons exist why autologous use is limited: firstly, there is a very slim chance of acquiring one of the few disorders currently treatable with UCB – i.e. ever having the need to use the stored UCB; and secondly in some cases, one's own UCB unit would be insufficient for transplantation purposes (Sullivan, 2008).

A unit could be deemed insufficient for autologous use because of the following main reasons: a) autologous units do not have the immunotherapy benefits of GvL, exhibited by allogeneic units that contribute in combatting leukaemia; b) pre-leukemic cells could be present in units of children who develop childhood leukaemia, thus rendering their UCB unit insufficient for transplantation; c) certain genetic disorders are transferred in the UCB SCs and these units can therefore not be used for autologous transplantation; these haematopoietic disorders which include hemoglobinopathies, inherited immunodeficiencies etc. can, however, be treated with allogeneic transplantation (Anon, 2006).

The WMDA estimates that "Approximately 70% of patients with blood disorders such as leukaemia, severe aplastic anaemia and congenital or other acquired disorders will not have a suitable family donor" (Anon, 2006). Siblings only have a 1:4 chance of being an adequate match whereas a 1:8 chance exists between a parent and child. However, with an adequate pool of publicly stored UCB units, the likelihood of finding an appropriate HLA-matched

allogeneic unit is at least 40% and increases as the number of publicly stored units increases (Anon, 2006).

5.2.3.5 Informing the patient of a unit's cell dose requirement

Private banks often tend to neglect to inform their clients about cell dose requirements needed for successful transplantation. UCB units often only yield cell numbers adequate to treat children and not adults (Paulin, 1992). In order to obtain successful engraftment, a unit should contain about 2.5 x 10^6 CD34+ cells/kg body weight of the individual who is to receive transplantation (Rocha et al., 2000; (Yang et al., 2005). One UCB bag (80-120ml UCB) generally contains enough stem cells (10x10^6 CD34+ HPCs) to successfully engraft a child of up to 4kg (Zhang et al., 2006). Thus, if a child is not diagnosed within the first three months after birth, the chances are that their single UCB unit would not contain enough SCs for transplantation purposes. For publicly banked units, this is not a problem, since HLA-matched samples of two unrelated donors could be pooled to overcome the issue of unit potency (Fong et al., 2012).

5.2.3.6 Banking for a nation…

Although the field of UCB banking is mostly polarised between the two seemingly opposing categories for storing UCB units (public or private), alternative models have been suggested to overcome these differences. It is generally agreed that UCB banks have a role to play in furthering future therapeutic applications of UCB, and that UCB units should be made available to the public. These two principles gave rise to so-called public-private hybrid UCB banks. There are many different models through which these hybrid banks operate, ranging from catering for both public and private banking to banks in which a certain percentage of each stored sample (e.g. 80%) is available for public access while the remaining sample volume (20%) is retained for private use (Jordaan et al., 2009). The last mentioned model is known as the Virgin model and is a rather controversial model in light of cell dose requirements discussed earlier (Martin et al., 2008). Hybrid bank models do not necessarily provide a steady solution to the on-going debates and probably contribute more towards current confusion and controversies. However, with advances in cellular therapy (e.g. induced pluripotent SC technology (iPS cells), cell expansion and tissue generation) there might be merit in investigating the benefits provided by these 'hybrid banks' in serving the public through both public and private storage of cell products.

It is important to have an intimate knowledge of the benefits provided by each of the above mentioned UCB models in order to best provide for the needs of South African citizens. A public UCB bank in South Africa would give many patients access to previously unavailable treatments by providing a large pool of genetically diverse UCB samples, representative of South African demographics. Given South Africa's genetic diversity and existent financial constraints for many citizens, there is no dispute that the country would immediately benefit from a public UCB bank.

5.3 Objective

The study presented here forms part of a larger feasibility study consisting of five components. Combined results from each of these components will determine the feasibility of establishing a public UCB SCB in South Africa. The final objective of the study presented here is, therefore, to establish whether there is public support for and interest in establishing a public UCB SCB (or banks) in SA.

The investigator's objective was thus to determine preliminary public support for the establishment of a public UCB SCB by addressing mothers attending the ante-natal clinic at the Steve Biko Academic Hospital in Pretoria. In addition to assessing public support, the investigator aimed to obtain information on potential elements that could impede the establishment of a public SCB. Some of these elements can be overcome and, when appropriately addressed, could have a negligible negative impact on public support for UCB banking. The major areas foreseen to potentially impact patient support were cultural and religious practices, language constraints, academic insufficiencies and patients' willingness to undergo additional HIV screening. Some of these elements (e.g. cultural or religious beliefs and practices that specifically have bearing on the patient's beliefs regarding blood, donations, tissues, body waste etc.) might not be dealt with in a practical manner as in the case of e.g. language constraints where a translator could be approached. If these elements were to pose significant concern they could considerably hinder the establishment of a public UCB SCB.

The results from this study should assist in the design of further more in-depth studies that must be conducted in different provinces across the country in public and private hospitals for a comprehensive overview of public support for UCB banking. The results could serve as proof of concept and the methodology could contribute to a more in-depth social-science-based protocol for addressing patients in clinics, hospitals and provinces throughout South Africa.

The investigators hypothesised that more than 50% of the public would support the establishment of a public UCB SCB in South Africa. They furthermore surmised that cultural and religious practices (related to blood donations) together with language constraints and academic insufficiencies of South African citizens might have a negligible impact on the establishment of a public human UCB SCB in South Africa.

5.4 Methodology

The objective of this public acceptability study was to gauge public reaction towards and support for UCB stem cell donation and banking, as well as to indicate which foreseen parameters could potentially impede this endeavour. Important parameters considered in addition to public support, were a patient's perception of the processes involved with UCB donation and banking and subsequent HIV testing (necessary for compliance with international regulatory standards).

An initial pilot study, involving 77 expectant mothers had been conducted previously (Meissner-Roloff et al., 2012), through which the current study's design and interview processes were optimised. Using the pilot study's refined template (Annexures 1 and 2), 217 randomly chosen, expectant mothers, attending the ante-natal clinic at the Steve Biko Academic Hospital were addressed during a 15 min. interview, followed by the completion of a closed anonymous patient questionnaire. Ethics approval for the study was obtained from the Main Research Ethics Committee at the University of Pretoria (protocol number: 131/2010) (Annexure 5).

Visual aids (a doll with an umbilical cord and placenta and relevant posters) were used to explain concepts relating to UCB banking during the interview. Participants were given the opportunity to raise questions and / or comment during and after the interview and were encouraged to write comments or questions in the space provided on the questionnaire itself.

These questions and comments were documented, together with data from the questionnaire, which was analysed using Microsoft Excel (Microsoft Corp., Redmond, WA). Confidence intervals were calculated with the help of biostatistician, Prof Piet Becker, using the Statistix program (Analytical Software, Tallahassee, FL).

The questionnaire was designed in a manner that would allow the investigator to obtain limited demographic information about the patients, while simultaneously addressing the issue of public support through a series of questions.

The demographic information was chosen for the following reasons and included:

A. <u>The patient's home language</u>

- In order to infer patient ethnicity (in an attempt to steer clear of potential racial connotations).

B. <u>Patient age</u>

- To investigate whether a patient's age could potentially impact on her understanding of and support for a public UCB SCB.

C. Number of biological children

- To determine whether prior experience with childbirth influences understanding of and support for a public UCB SCB.

D. Marital and employment status

- Considered to infer the potential influence that emotional and financial support of the individual has on her understanding of and support for a public UCB SCB.

The following six questions were designed to obtain information about patient support for a public UCB SCB and to ascertain the interviewee's understanding of associated UCB SCB processes gained from the interview:

A. Question 1 (Q1):

If there is a public cord blood bank facility, would you be willing to donate your PLACENTA (afterbirth) for medical research?

If the participants answered NO to Q1, they were asked to provide one of the following reasons for not being willing to donate:

Q1 Reason:

- Against religious belief
- Against your culture
- Don't think this bank is a good idea
- Afraid of the collection process
- Don't understand what the bank is for
- Other (please specify)

B. Question 2 (Q2):

If you answered NO in question 1, would you be willing to donate the BLOOD from your placenta?

Question 3 (Q3):

If you are willing to donate your placenta OR just the blood from the placenta and umbilical cord, would you be willing to allow your doctor to do an additional HIV test?

C. Question 4 (Q4):

Have you heard of "stem cells" before today?

D. Question 5 (Q5):

Do you think stem cells can help to treat you, your child or somebody else in the future?

E. Question 6 (Q6):

Do you think that a public umbilical cord blood stem cell bank is a good idea?

5.5 Results and discussion:

5.5.1 Introduction

Although a few routine medical procedures exist that involve UCB transplantation, there are many more possibilities for potential treatments that could be explored in the future. With vastly improving techniques for expanding haematopoietic stem cells (HSC) in culture, it is possible that samples stored in public banks will become a vital resource for novel forms of therapy in the future. It was therefore deemed beneficial to include in the questionnaire the possibility that samples could be used for medical research and / or public use. The term "medical research" was used to describe all downstream applications that involve current and possible future treatments with UCB. A clear distinction was, however, made during the interview process between the current use of UCB for transplantation and research purposes.

Many of the mothers-to-be attending the antenatal clinic at the Steve Biko Academic Hospital had high-risk pregnancies and were often referred by their local clinics. It can be argued that these mothers had better access to and received more information regarding their pregnancies and might thus have been more educated about their pregnancies than mothers attending other clinics. This could have facilitated the presentation of information to the mothers at this clinic, and it was understood that it might be more difficult to convey the same information in rural clinics.

5.5.2 Results and discussion for Question 1 to Question 6

Overview

Despite the interviewer's efforts, questions were sometimes left unanswered (BL), which delivered 'non-workable data' (non-useful data). Patients that left some questions unanswered could have done so for various reasons: It could either be an indication that the patients did not understand the question, were undecided or reluctant to answer (e.g. to undergo an additional HIV test) or were not able to complete the questionnaire because of time constraints (e.g. called by a nurse or doctor).

In order to obtain about 200 questionnaires with workable data (i.e. not left unanswered) the investigator interviewed 217 patients (to replace non-workable data with workable data). Figure 4 gives an overview of all the results obtained (useful and non-useful) for questions one to six (Q1 to Q6).

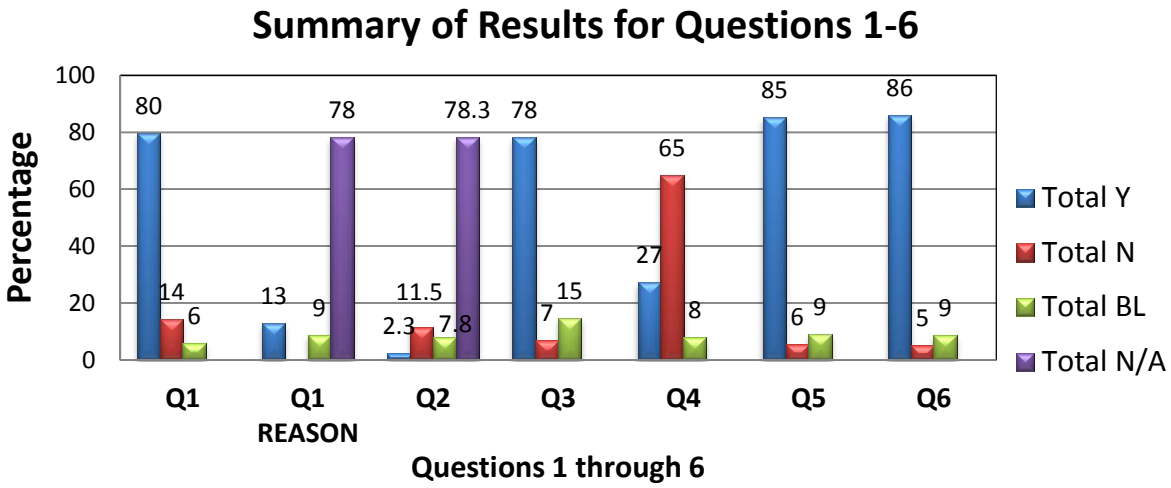

Figure 4: Summary of results obtained from questionnaire, Questions 1-6. Answers were either indicated as "Y" (yes) – indicated by the blue bars; "N" (no) – indicated by red bars; "BL" (left blank) – indicated by green bars or "N/A" (not applicable) – indicated by purple bars. *Note: All selected Q1 Reasons were considered as a positive response and therefore categorised under "Yes", to illustrate that reasons were provided as opposed to being left blank or "N/A".

Figure 5 indicates the ratio of useful data gathered for each question versus non-useful (blank or unanswered) data. Figure 5 shows that more that 85% of all the gathered data was useful and could be used for downstream analyses.

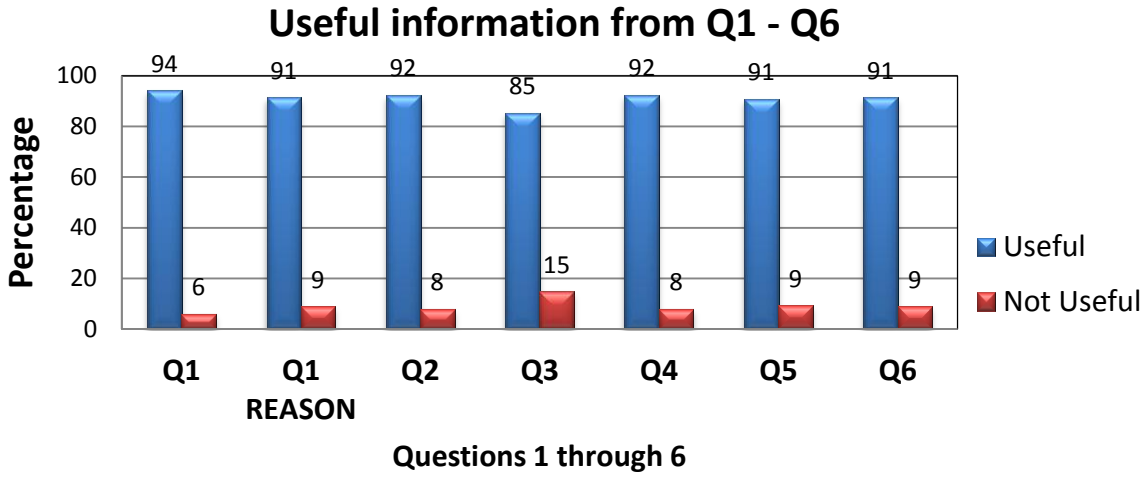

Figure 5: All answered questions were deemed 'workable data' or 'useful' and are represented above for Questions 1 through 6 (Q1 - Q6). Workable (useful) information (blue bars); blank or not useful information" (red bars).

5.5.3 Results for Q1

Q1: "If there is a public cord blood bank facility, would you be willing to donate your PLACENTA (afterbirth) for medical research?"

Support for donation of the placenta was measured in Q1 – where 80% of participants were willing to donate their placenta (Fig.7). In order to infer the reliability of this result, a 95% confidence interval (CI) was calculated. Using the Statistix software, the interval was calculated as [74.4% to 85.1%]. When results were corrected to use workable data only, the number of patients supportive of Q1 increased to a liberal 85% (Fig. 7) (95% CI of [79.9%, 89.7%]).

Patients unwilling to donate their placenta were asked to provide reasons for their reluctance in order to ascertain whether a particular concern was dominant amongst the group. Patients were provided with a list of potential concerns and were allowed to indicate more than one concern/reason for their reluctance to donate their placenta. These concerns included any of the following reasons or combinations of these reasons. Results are illustrated in Figure 6.

Q1 Reason:

1. Against religious belief
2. Against your culture
3. Do not think this bank is a good idea
4. Afraid of the collection process
5. Do not understand what the bank is for
6. Other (please specify)

It can be seen in Figure 6 that the majority of patients (78% N/A) indicated no reasons for personal concern or unwillingness to donate to a public SCB. It corresponds to the 80% of patients that were willing to donate their placentas in Q1. The discrepancy between Q2's 78% (Fig. 6) and Q1's 80% (Fig. 7) can be attributed to two patients that were willing to donate their placenta in Q1 but still gave reasons why they might not be willing to donate their placentas. It is unclear why these patients also indicated reasons for unwillingness to donate their placentas and raises the question as to what extent these two patients understood the presentation and subsequent questionnaire questions. Upon investigation of answers provided for the remainder of the questionnaire, although the first patient was willing to donate the placenta and the blood from the placenta, she indicated that donation of the placenta might be against her religion. The remainder of the questionnaire was unfortunately left unanswered and the

investigator cannot make any definitive conclusions with regard to the patient's understanding of the presentation or banking as a whole. The second patient was willing to donate the placenta but not the blood from the placenta and indicated her reason as "it is sometimes not safe". This patient left Q3 unanswered, but was otherwise supportive of establishing a public UCB SCB.

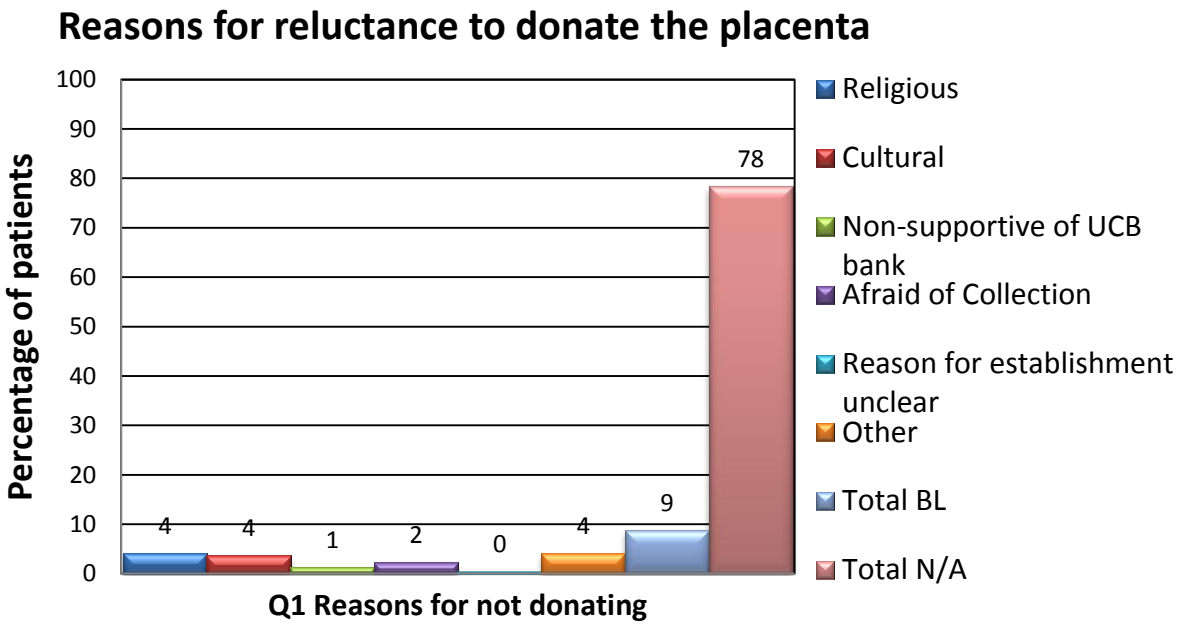

Figure 6: Question 1 Reasons: Patients' reasons why they would not support a public UCB SCB. Patients that answered "NO" to Q1 were asked to indicate why there were reluctant to donate their placentas. The investigator provided the following options: a) Against religious belief (dark blue bar); b) Against your culture (red bar); Do not think this bank is a good idea (green bar); Afraid of the collection process, (purple bar); Do not understand what the bank is for (turquoise); and Other (orange bar). Unanswered "Reasons" – (left blank) are indicated by the light blue bar. Patients that answered "YES" to Q1 were instructed to write "N/A" (not applicable) (pink bar).

No specific problematic areas were observed that related to patients' reluctance to donate their placenta. The reasons provided by the 22% of unsupportive patients were varied and not linked to a specific language group (discussed later). There was an equal amount of concern that donation of the placenta might be against people's religion or culture (4% respectively) while 4% also provided their own reasons for their reluctance to donate their placentas (Fig. 6). Only 1% of people were unsupportive of the idea of a public UCB bank while 2% were afraid of the collection process. It also seems that everybody understood the reasons provided by the investigator for establishing a public UCB SCB in SA, since nobody indicated the reason for their reluctance to be an unclear understanding of the motivation for establishing an UCB bank (Fig. 6).

Personal reasons provided by 4% of the patients were mostly concerned with fears unrelated to reasons provided on the questionnaire. These personal reasons included: 1) fears that collection procedures could be unsafe; 2) the study overwhelmed the patient and created uncertainty; 3) there was reluctance because of the person's HIV status (two individuals); 4) the person suffered from epilepsy and was afraid that the blood would not be used for intended purposes 5) – "it is part of somebody's body" 6) patient was not interested; 7) fear of donation.

These concerns could be addressed by assuring the patients of the safety and efficacy of the UCB collection, banking and redistribution procedures. Once UCB banking becomes common practice, people with these fears might feel less intimidated by the "novelty" of UCB technology while others might become more supportive once the positive effects of UCB transplantation become known in the community after successful treatments.

The investigator could not make any inference from the results that religious or cultural concerns (related to blood, blood donations etc.) were more prominently associated with a specific language group (or implied ethnic groups) for two reasons: (1) results displayed might not hold true for different demographic settings in South Africa; although the patient cohort was diverse, some ethnic groups – who might still have cultural, religious or other objections - were underrepresented; (2) the questionnaire asked the patients to indicate their first language and not their ethnicity, culture or religion; in trying to steer clear of any racial insinuations, the investigator wrongly assumed that language could be a good indicator of the person's ethnicity, culture or religion, which is not the case, as discussed later.

For these two reasons it is most likely premature to conclude that religion and culture could not significantly impede the establishment of a public UCB SCB, although the influence of religion and culture in our study cohort seems to be negligible. It is important to consider that their influence might be more pronounced in rural areas where people might adhere more to their customs, or amongst different ethnic groups not adequately represented in our patient cohort.

Unfortunately all of these reasons are conservative estimates for reasons mentioned above, since 9% of the participants left the reasons blank or unanswered, which, therefore, does not exclude them from not having cultural, religious or other more personal concerns.

Question 2:

Q2: "If you answered NO in question 1, would you be willing to donate the BLOOD from your placenta?"

Patients were requested to answer Question 2 only if (1) they answered "No" to Q1 or (2) if they were of a cultural or religious group that would, under normal circumstances, approach their physician with the request to take the placenta home after birth. This would give an estimate of patients that might support UCB banking, but because of cultural or religious practices involving the placenta, might oppose donation of their placenta. The investigator thus wanted to determine whether these patients – that were unwilling to donate the placenta – would be willing to donate the blood from the placenta and thereby still support UCB banking.

All the workable/useful data for Q2 is indicated in Fig.7. From these results it can be seen that of the initial 16% of patients who were against donation of the placenta, 3% (2.5%) were willing to donate the blood from the placenta, 13% (12.5%) were not willing and answered "No" to Q2, while 85% of the participants indicated that this question was not applicable to them (comparing well with results from Q1, Fig.7).

The 3% of patients willing to donate the blood from the placenta (thus answered "Yes" to Q2) can be divided into the following groups:

1) Patients that left Q1 blank but answered "Yes" to Q2:

   Account for 1% of the 3% who said "Yes" to Q2.

These patients were willing to donate the blood from the placenta. One of these mothers indicated that it is against her culture to donate the placenta.

2) Patients that answered "No" to Q1 but said "Yes" to Q2:

   Account for 1% of the 3% who said "Yes" to Q2.

This could indicate that these patients might want to retain the placenta, but would allow the blood to be collected from the placenta for UCB donation. One patient indicated that her reluctance to donate the placenta was due to her HIV-positive status, although this did not influence her decision to donate the blood from the placenta. A misperception that the investigator encountered a number of times during the patient interview was that some patients believed that blood "outside of the body" does not contain HIV anymore and would

thus not be able to infect people. It is probable that this perception arose from misinterpretation of safety guidelines for dealing with HIV. Although it is true that the HIV virus is fragile and does not survive well 'outside of the body', i.e. when exposed to air, heat or other chemicals, it should clearly be distinguished from collected body fluids – such as donated blood, where the HIV virus remains viable.

3) One patient (1%) answered "Yes" to Q1 and "Yes" to Q2.

This patient filled out both Q1 and Q2, although it was not necessary for her to complete Q2 (based on her answer in Q1). She was willing to donate both the placenta and the blood from the placenta.

Of the 13% that answered "No" to Q2, 12% were neither willing to donate the placenta (Q1) nor the blood from the placenta (Q2) while the remaining 1% left Q1 unanswered. Upon investigating the reasons for these patients' reluctance, it was found that 3% of these patients indicated that donation was against their religious belief while 2% indicated that it was against their culture (Table 1).

Table 1: Reasons why patients were reluctant to donate either the placenta or the blood from the placenta

Q1 Reason for not donating placenta	Number of patients indicating each reason
Against religious belief	6 (3%)
Against culture	4 (2%)
Bank is not a good idea	1
Afraid of the collection process	4 (2%)
Don't understand reason for the bank	0
Other (2%)	1 (Want to think about it) 1 (HIV status) 1 (Placenta is a part of somebody's body) 1 (Not for me)
Blank	6 (3%)

Note: Patients could supply more than one reason

Question 3:

Q3: "If you are willing to donate your placenta OR just the blood from the placenta and umbilical cord, would you be willing to allow your doctor to do an additional HIV test?"

Of all the questions, Q3 was left unanswered most often. Figure 7 indicates that 85% of data gathered for Q3 was useful, while 15% was left blank/unanswered. Figure 4 illustrates that 78% of patients were willing to undergo additional HIV testing, 7% were not and 15 % left the question unanswered.

The reasons for the patients' reluctance to answer this question are uncertain. Patients might have been uncertain about the need for a second HIV test since all attending patients at the antenatal clinic in the Steve Biko Academic Hospital had already undergone HIV testing. However, the investigator made these reasons clear during the interview. It is more likely that patients might have been fearful of undergoing additional HIV testing. Since mothers presumably know their status because of the previous HIV screening, fears could be related to either being exposed as being HIV positive or a fear that their status might have changed from negative to positive.

It is also possible that some of these patients weren't comfortable with needles and didn't want to go through the process of testing again while some of the patients could potentially not have been able to complete the entire questionnaire and therefore left this question unanswered. Although much has been done in South Africa to overcome the problems of stigmatisation because of an individual's HIV status, there are still many who hold views and fears that reveal stigma. When the results were corrected to look only at useful data, 92% of patients were willing to undergo the additional HIV test, while 8% were unwilling (Fig. 7).

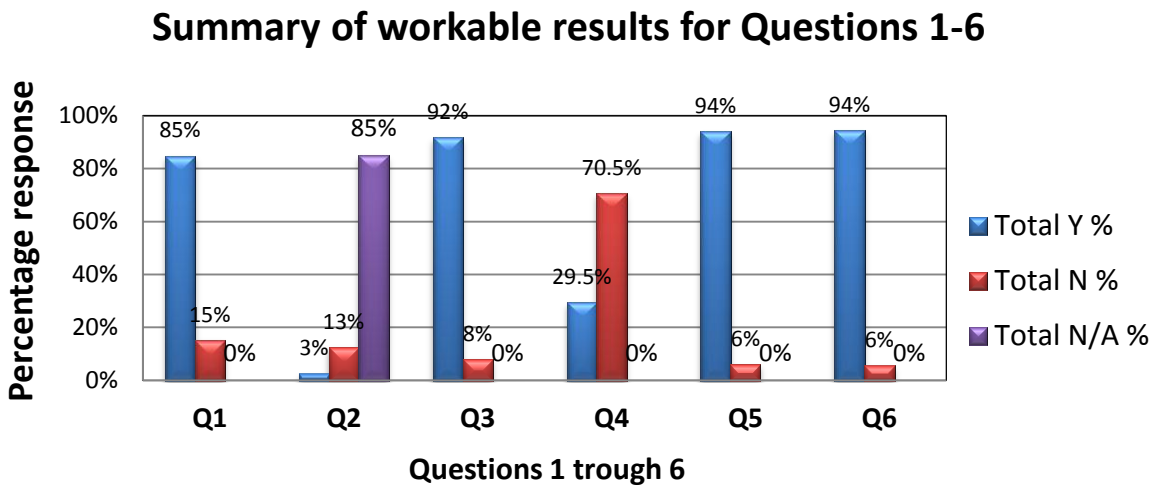

Figure 7: Summary of useful information obtained from Questions 1 through 6 (Q1 to Q6). Questions answered by "Y" (yes) are indicated by blue bars; "N" (no) indicated by red bars and "N/A" (not applicable) indicated by purple bars.

Since it is a prerequisite to test all UCB prior to banking, a person's refusal to undergo an additional HIV test would result in the donated UCB being discarded. It is therefore important to ask how many of the patients that were willing to donate their placentas (Q1) were also willing to undergo the additional HIV test (Q3). Without taking the unanswered questions into consideration, it was found that 71% of patients indicated that they were willing to donate their placenta and undergo an additional HIV test.

Question 4:

Q4: "Have you heard of stem cells before today?"

and

Question 5:

(Q5):"Do you think stem cells can help to treat you, your child or somebody else in the future?"

Question 4 was intended to serve a dual purpose: 1) to verify whether prior knowledge about stem cells and UCB SC banking could influence the patients' readiness to donate; and 2) when analysed together with Q5, to be used as a crude measure of the patients' understanding of the concepts discussed in the presentation; i.e. a person that did not know what stem cells were before the presentation (Q4) but understood that SCs could be used to treat patients with certain diseases (Q5) after the interview, presumably understood the content of the interview.

Figure 7 illustrates that almost 30% of patients had heard of stem cells before being introduced to stem cells during the interview. This number is surprisingly high and might be due to a misunderstanding of the question. Before starting with the interview, the investigator would ask the patients how many of them knew what stem cells were. The investigator's observation was that far fewer than 30% of patients knew what stem cells were, with the true number being closer to 10% to 15%. It could be argued that some patients with prior knowledge about stem cells were reluctant to raise their hands in answer to this question at the onset of the presentation, for fear of being singled out. However, during some of the one-on-one interviews, the investigator encountered a misunderstanding of the question: some patients understood the question to mean "have you heard of stem cells today" and not "BEFORE today". This could mean that patients did not always read the whole sentence or that some might have had trouble comprehending the question, which could be attributed to language constraints.

These problems could be addressed by clarifying the question or by putting it first in the questionnaire and allowing patients to answer it before the start of the presentation.

For a more direct measure of the patients' understanding, this question could in future be phrased to ask for direct feedback e.g. "what are stem cells?" or "what can stem cells do". Given the current language constraints, this might, however, complicate the questionnaire and might only be valuable if the questionnaire and interview could be translated into different languages to facilitate better understanding.

Figure 7 furthermore indicates that 94% of patients were of the opinion that SCs can be used to treat people with certain haematological diseases (Q5). This is very encouraging, since at least 70% of the patients were unaware of SCs before the presentation (Fig. 7, Q4) and did not know anything about their therapeutic application beforehand. It stands to reason then, that these patients understood the content of the presentation, which enabled them to answer in the affirmative – that SCs can be used therapeutically.

The investigator was of the opinion that having prior knowledge about SCs would be beneficial to obtaining public support for a SCB. Although this might be true in cases where patients are not thoroughly informed during the informed consent process, it does not seem to significantly impact patient support when adequate information is presented to the patients. The impact of prior knowledge (Q4) on a patient's willingness to donate their placenta (Q1), their understanding of elements presented during the interview (Q5) and support for UCB SC banking (Q6) are illustrated in Figure 8. These results highlight the importance of the "informing the patient" component when obtaining informed consent. When patients feel empowered by the knowledge presented and are not pressured into making decisions about concepts that they feel uncertain about, there are seemingly few deterrents to obtaining patient support.

Although a remote possibility in our particular context, it could also be argued that patients that had previously heard of SCs might have had their own reservations based on what they had heard. Controversies related to embryonic stem cells (ESCs) are frequently reported in the public media. If these patients had been introduced to controversies related to ESCs, they might have been more reluctant to donate their UCB because of the confusion. This might be a more plausible explanation in countries where the ESC debate has been more pronounced,

such as the USA. However, in comparison to the USA, South Africa has had limited public exposure to the ESC versus adult SC debate, rendering this possibility rather unlikely.

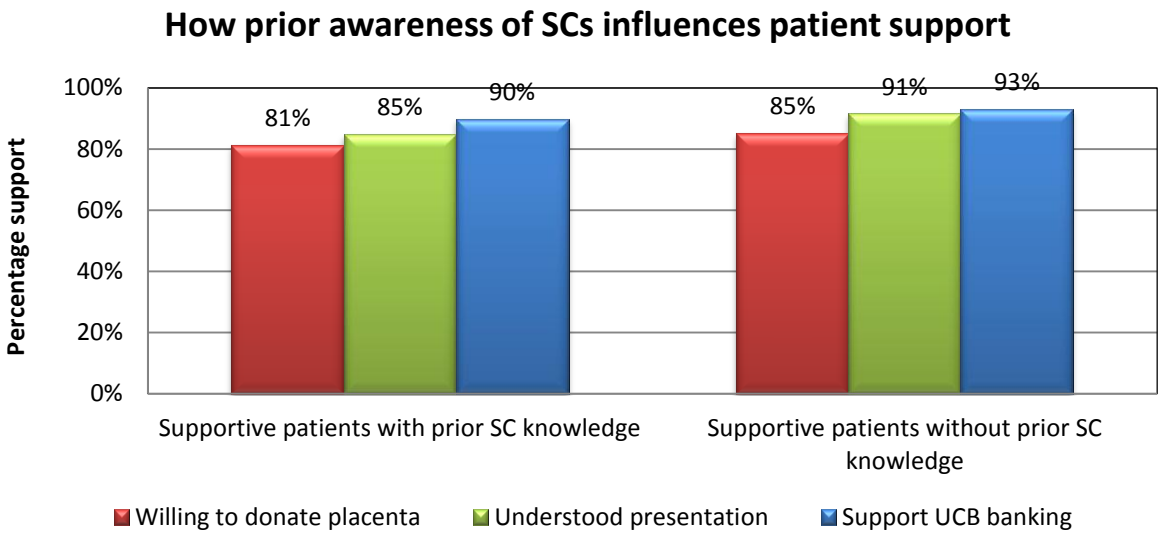

Figure 8: Illustrates how prior knowledge about stem cells influences patient willingness to donate the placenta (Q1) (red bar); their understanding of the presentation (Q5) (green bar); and their support for a public bank (Q6) (blue bar).

Question 6:

(Q6): "Do you think that a public umbilical cord blood stem cell bank is a good idea?"

This question provides direct information regarding the patients' support for the establishment of a public UCB SCB. As mentioned earlier, it could furthermore serve as a crude measure of patients' understanding about the presentation and thus processes related to UCB SC banking.

Together with Q5, these two questions received the most positive responses from the patients, with 94% (95% CI, [91.3.% to 97.6%]) of patients being supportive of establishing a public UCB SCB (Fig. 7, Q6) (a liberal estimate since it does not take blank / unanswered data into account). It should be noted that this result differs from patients that were willing to donate their placentas in Q1, where only 85% of patients were willing to donate their placentas (Fig. 7). There thus seems to be higher theoretical support for donation than actual support.

This 9% discrepancy can be attributed to the following reasons: 4% of these patients indicated that donation was either against their own cultural or religious beliefs (2% respectively) but were still supportive of the idea of banking; 2% indicated that they were afraid of the donation process involved in Q1; 2% left the reason for their reluctance to donate their UCB blank while

the remaining reasons are attributed to personal reasons. Some of these personal reasons indicate that a few patients were unwilling to donate the placenta themselves, although they were theoretically supportive of establishing a public UCB SCB. They indicated reasons such as that they would "like to think about it" or "was taken by surprise".

It might be unrealistic to think that 85% of patients would follow through with their decision to donate the placenta. The questionnaire was theoretical and very little is at stake. Once confronted with the actual informed consent documents, these patients might not all be as willing to donate their placentas to medical research. However, results presented here are very encouraging and seem to provide more than sufficient public support for establishing a public UCB SCB, provided that patients are given adequate information in order to make an informed decision.

5.5.4 Other patient information

As mentioned previously, the investigator included a section to collect information regarding patient demographics. These demographics included: patients' language, age, number of biological children, their marital and employment status. The influence of each of these demographics is explained below:

Patient language:

In order to obtain an indication of how language constraints influence the patients' understanding of the presentation as well as their support for UCB banking, patients were asked to indicate their first language.

Although the patient cohort was diverse, many of South Africa's 11 official languages were underrepresented as can be seen in Figure 9. This is mostly due to the location where the study was performed and it therefore needs to be repeated in different provinces throughout South Africa where different language distributions occur. Of interest is that some patients attending the clinic were from neighbouring countries and have either immigrated to South Africa or work in South Africa but still hold different nationalities. One patient from North Africa accounts for the French indicated in Figure 9.

Although some useful information could be gathered from the indicated languages and the corresponding answers to the questionnaire, it does not provide a complete picture of the language constraints present. Since filling out the questionnaire was voluntary, there were

some patients that were unwilling to fill out the questionnaire. Of these patients, it was evident that some were unable to understand anything during the presentation because they could not understand the language spoken (English). Their English was at best only broken English and these patients would have benefitted most from a translated questionnaire and interview (or a translator). Some were however, not interested in participating and declined to answer the questionnaire. Therefore, results obtained for the influence of patient language on the patients' understanding of the questionnaire is significantly skewed towards patients that were able to comprehend.

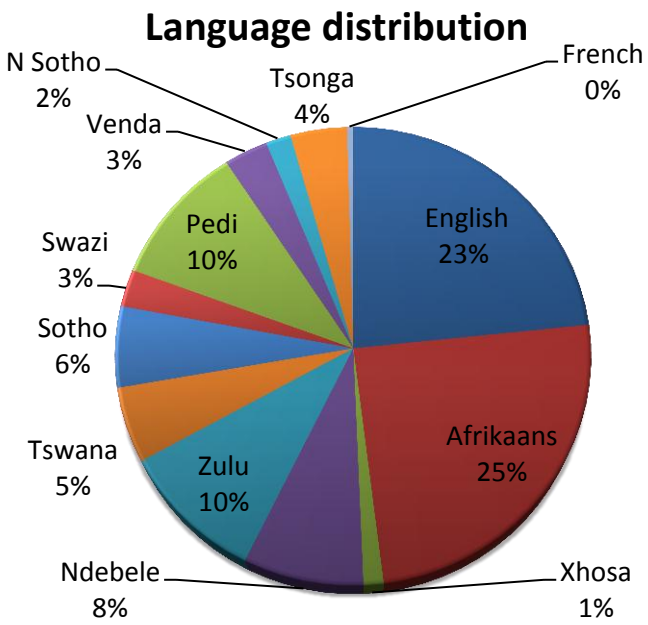

Figure 9: An illustration of the language distribution of participating patients. Although the patient cohort was diverse, all language groups were not adequately represented.

Unfortunately the investigator did not annotate the initial numbers of patients in the audience and the number of patients that were clearly unable to understand the presentation due to language constraints. These elements (audience size vs. actual participation) are extremely important parameters, which could provide useful information about public understanding and subsequent support. This information needs to be added to the final protocol if used in a national survey of patient support of an UCB SCB.

Figure 10 illustrates how language could potentially impact on a patient's understanding of the presentation. It should also be noted that it does not account for patients that could not participate in the questionnaire due to a complete lack of understanding. Therefore, these

numbers are an overestimate of the true situation. Many language groups were underrepresented; therefore, Figure 10 illustrates the proportional relationship of the most frequently encountered languages to the patients' understanding of the questionnaire (Q5), while the lesser-encountered languages were grouped together under "Other".

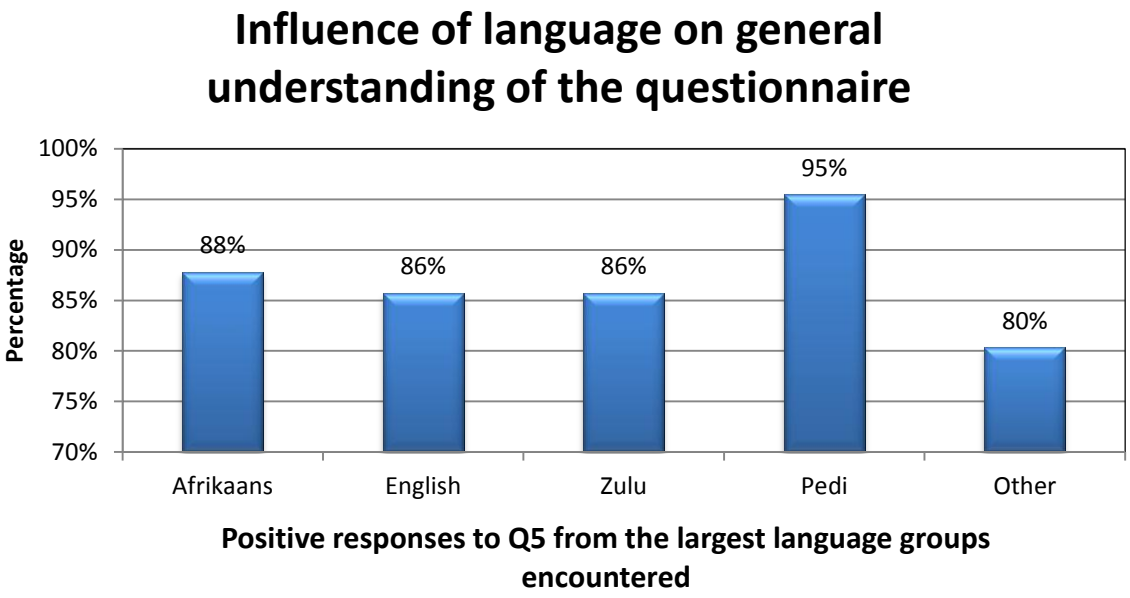

Figure 10: The influence of language on the patients' understanding of the questionnaire (Q5).

To facilitate a better understanding of the interview and questions in the questionnaire, the investigator encouraged discussions amongst attending patients. Often, a patient with sufficient English comprehension would translate some of the patients' questions to the investigator and *vice versa* in order to facilitate a better understanding among the patients. However, it must be assumed that most of the patients that completed the questionnaire had at least a fair understanding of English or Afrikaans (the two languages spoken by the investigator and used to clarify concepts and answer questions).

Results illustrated in Figure 10 seem to indicate that language does not significantly impede the establishment of a public UCB SCB. It is however an overestimation and confirms the importance of properly addressing the issues concerned in UCB banking through easily accessible language understandable to all patients. In the opinion of the investigator, this will be the single most important defining factor in influencing patient support throughout SA for the establishment of a public UCB SCB.

<u>Language is not a sufficient indicator of ethnicity, religion or culture</u>

As mentioned previously, the investigator wrongly assumed that language could simultaneously provide information regarding a patient's understanding of the questionnaire, as well as serve as a good indicator of a person's ethnicity, religion or culture. This is unfortunately not always the case; e.g. an Italian (ethnicity) born in the USA would probably speak English (language), thus this mistake renders information regarding patient ethnicity insufficient.

Although it holds true that sometimes a person's first language could be an indication of his ethnicity, it was found that many South African mothers-to-be customarily adopt the language spoken by the husband as their home language. Patients might have indicated this home language instead of their own first language and this does therefore not necessarily imply a person's ethnicity and states nothing about a culture or religion. Thus, if the husband speaks Zulu and the patient Pedi, their home language would most likely be Zulu while the patient's ethnicity could be Pedi.

Furthermore, many African patients often speak more than one African language and in some cases neither husband nor wife speaks their partner's first language. In these situations they communicate in a second language that subsequently becomes the home language.

Therefore, all inferences on relationships based on language groups (as a substitute for ethnic, religious or cultural groups) are at best a crude indicator of the influence of ethnicity on patient support and understanding of UCB banking. An example of this is found in Figure 9 where 23% of patients indicated that they spoke English while 25% of patients spoke Afrikaans. This does not indicate whether these patients were Caucasian English or Afrikaans-speaking patients, were Coloured, Indian or of African origin. While 25% of the patients were Afrikaans speaking, at most half of these were Caucasian (investigator observation) while the rest constituted Coloured and only a few African patients. In contrast, very few Caucasian English-speaking patients took part in the study, while the majority of the 25% of English-speaking patients were of Indian or African origin (investigator observation).

It will be important to establish women's cultural practices in relation to body waste (i.e. placenta) in pregnancy, as well as to tissues, blood and donation or "banking". Therefore, suffice it to say that more accurate information regarding patient ethnicity, culture or religion needs to be obtained through an in-depth and systematic study. Without this, no conclusions can be drawn about cultural or religious practices related to certain ethnic groups or more support for UCB banking from specific ethnic groups.

Influence of patient age on support for a public UCB SCB

From observations made during the pilot study (which preceded the current study), the investigator observed that younger patients seemed to be more supportive of UCB banking than older patients. In order to better quantify this observation, the questionnaire was revised to make provision for annotating patient age.

The majority of patients that attended the clinic and took part in the survey were between the ages of 26 to 30 (Figure 11). However, when patients were grouped into "younger" (ages 18 to 30) and "older" (ages 31+) groups, the numbers were almost equal, with 112 younger patients and 93 older patients (the remaining 12 patients did not indicate their age). Results, corrected for the number of patients per age group are indicated in Figure 12 and show how the patients' age affected their willingness to donate their placenta (Q1) and their support for a public UCB SCB (Q6).

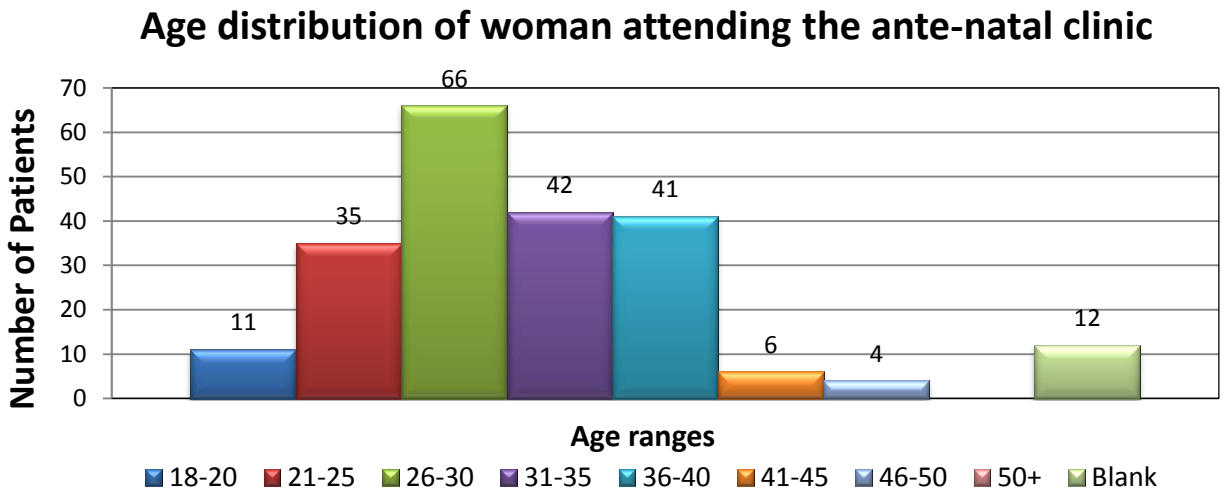

Figure 11: Age distribution of patients attending the Steve Biko Academic Hospital's antenatal clinic. Ages ranged from 18 years to above 50.

Of the younger patients, 81% indicated their support for both Q1 and Q6, while 70% support was obtained from the older patients. Assessing each question individually, younger patients were more willing to donate their placentas (84%) than older patients (77%) and younger patients were generally more supportive of the idea of establishing a public UCB SCB (92%) than older patients (82%) (Figure 12). To establish whether this observed difference in support between the age groups could be due to a difference in understanding of the presentation, the patients' ages were compared to their understanding of the questionnaire (Q5) (results not shown). It was found that 90% of the younger patients understood the information presented

and thought that SCs could be used to treat people with certain disorders. The older group's understanding (81%) corresponds well to their willingness to support the bank (82%) and could potentially imply that their weaker comprehension had a greater impact on their support than the younger group with better comprehension.

Other possible reasons that could explain greater support from younger patients include: 1) that younger patients have been more exposed to the latest technology. As a consequence, they might be more open to acceptance of new technological concepts, might be less intimidated by these concepts and more keen to explore new areas of innovation; 2) younger patients could be better equipped (schooling and thus comprehension) than their predecessors; 3) older generations could potentially be more reluctant to participate based on beliefs shaped by previous political regimes.

However, most patients that took part in the study seemed to adequately understand the presentation regardless of their age. It seems that the determining factor for obtaining support is to equip the patients with adequate and accessible information in order to make a properly informed decision. This information should be tailored to address not only people with different levels of schooling, but should also be 'age friendly'; i.e. should accommodate older people's lack of understanding of technological development and comprehension.

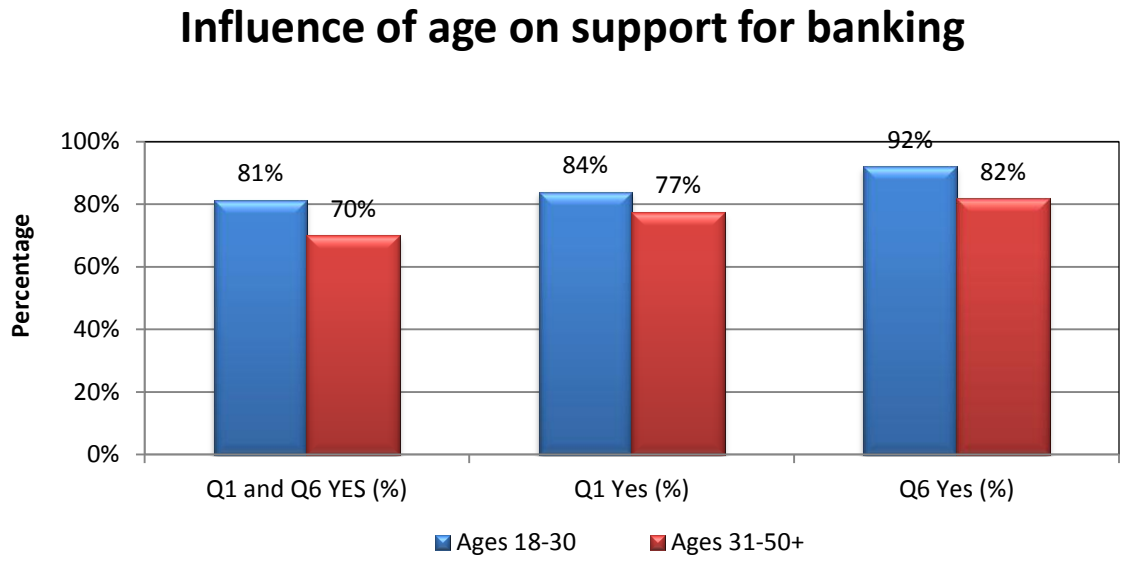

Figure 12: The influence of age on the support for establishing a public UCB SCB. Patient ages were grouped into two groups: "Younger" between the ages of 18 and 30 indicated by the blue bars, and "Older", above the age of 30, indicated by the red bars. Q1 tests a patient's willingness to donate the placenta, while Q6 tests the patient's support for establishing a public UCB SCB.

The influence of exposure to childbirth on patient support

Another factor that could influence a patient's understanding of UCB banking and related processes (discussed during the presentation) could be a patient's prior exposure to childbirth. During the pilot study (mentioned earlier), the investigator encountered patients who were unsure of what a placenta was. The word was subsequently translated in order to clarify its meaning but some patients were still unsure of the placenta's role during pregnancy and its normal disposal after pregnancy. It was therefore thought that a patient who had gone through the process of childbirth would know what to expect, understand more, be less afraid of donation and would subsequently be more supportive of a public bank. Figure 13 and Table 2 illustrate the number of children born to mothers of different age groups.

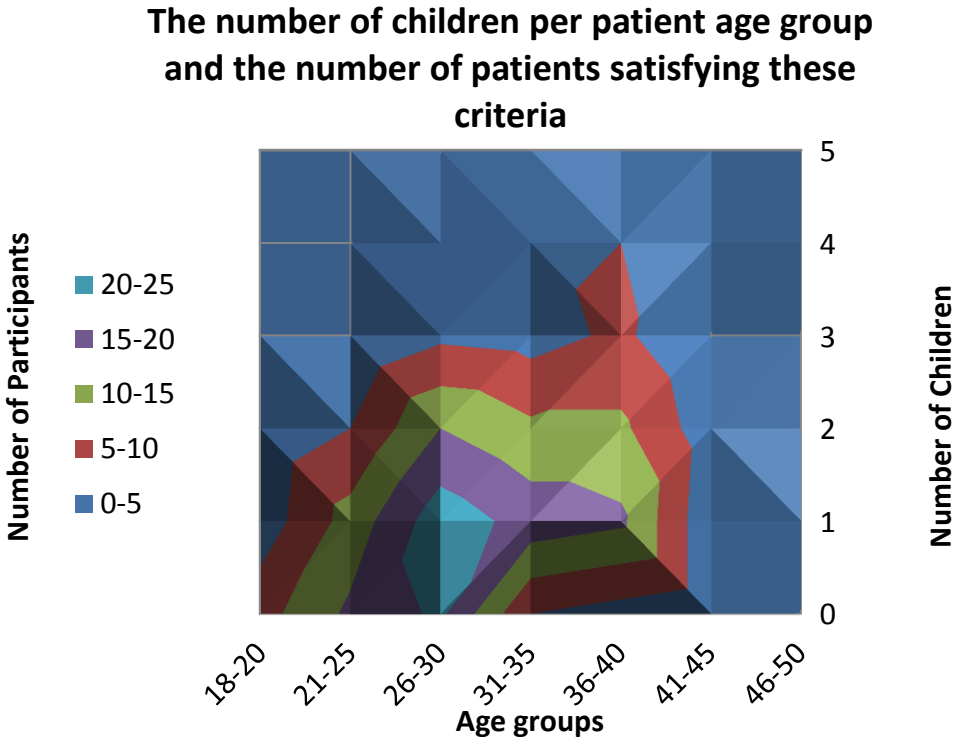

Figure 13: Indicates how many patients (indicated in colour and contour) accounted for both criteria: i.e. the a) number of children per b) patient age group. The largest group of patients (20-25 patients) is indicated by the turquoise area, followed the purple (15-20); green (10-15); red (5-10) and blue (0-5) groups.

It can be seen in Figure 13 that 20 to 25 patients (turquoise area) had one child and were between the ages of 26 and 30. Table 2 illustrates these numbers. The second highest prevalence for these categories was the 21 patients between 26 and 30 years without any children.

Table 2: Number of children for patients in different age ranges

Age	Number of Children					
	0	**1**	**2**	**3**	**4**	**5**
18-20	8	2	1	-	-	-
21-25	16	12	5	-	-	-
26-30	21	23	15	4	2	-
31-35	5	18	11	3	4	1
36-40	3	16	11	6	5	-
41-45	1	1	2	-	1	1
46-50	-	1	-	-	1	1

Patients were grouped into two categories: Patients with biological children and patients without biological children. There were 54 patients without any children and most of these (45) were below the age of 30. When comparing support from these two groups (with and without children), it seems that patients without children are slightly less supportive of establishing a public UCB SCB.

Results illustrated in Figure 14 reveal that 84% of patients without children were willing to donate their placenta (Q1), 90% of them thought that SCs could be used to treat patients (Q5) and that building a public UCB SCB is a good idea (Q6) respectively. Patients with children responded similarly but with somewhat greater support for these questions, with 85% of patients willing to donate the placenta (Q1), 95% understood the application of SCs (Q5) and 96% thought establishing a public UCB SCB is a good idea.

Collectively, all three questions related to support and understanding of a public UCB SCB (Q1, Q5 and Q6) indicated 80% support from patients without children and 85% from patients with children. In each case, it seems that having gone through the experience of childbirth (or previously being exposed to it) slightly aids the patient's understanding of the concepts involved in UCB banking – e.g. what the placenta is; what it does during pregnancy; that it is discarded after pregnancy, etc.

These patients might subsequently be less fearful of the unknowns associated with UCB collection than patients without childbirth experience. Nevertheless, the results are very similar between the two groups and the differences are not statistically significant. Although

childbirth exposure could potentially influence patients' support and understanding of UCB banking, it is unlikely to significantly impede the establishment of a public UCB SCB.

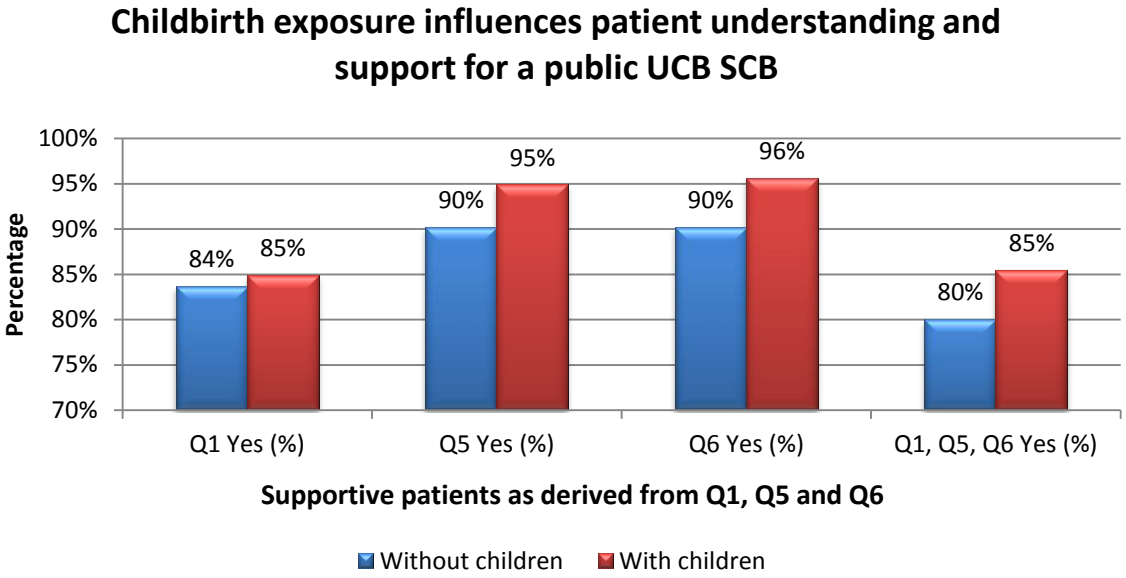

Figure 14: Exposure to childbirth influences patient of understanding and support for UCB banking. Results displayed indicate positive answers ("Yes") to Q1, Q5 and Q6 for patients with children (red bars) and without children (blue bars) respectively.

Patient demographics: Marital status and employment status

These two parameters provide personal information about patients by shedding light on their current circumstances and potential support structures. The possible relations between marital status, employment status and patient support are far too numerous to be adequately analysed with the few parameters provided by this survey. However, they could be used as initial probes into whether marital or employment status has any impact on patient support whatsoever, which could merit a more comprehensive analysis of potential contributing factors for each category.

Figure 15 indicates that the majority of patients (47%) were married (M), followed by 44% unmarried patients (U). The remaining patients were either widowed (W) (1%), divorced (D) (2%) or left the category unanswered (BL) (6%).

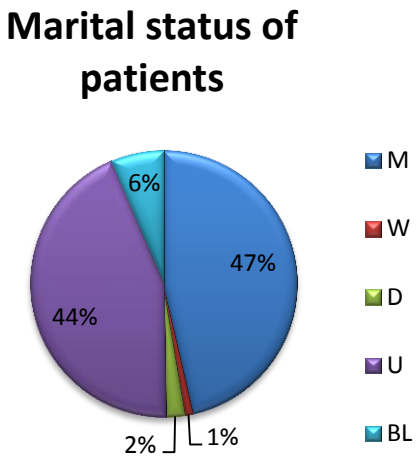

Figure 15: Marital status of patients that partook in the survey. Marital status was indicated to be: Married (M, dark blue); Widowed (W, red); Divorced (D, green); Unmarried (U, purple) or were left unanswered (BL, light blue).

Information gathered on patient employment is illustrated in Figure 16. It indicates conservative estimates of 48% unemployment (U) and 31% employment (E), since 21% of patients left the question unanswered (BL).

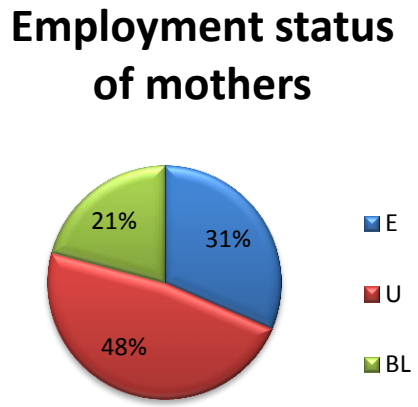

Figure 16: Marital status of patients that partook in the survey. Marital status was indicated to be: Married (M, dark blue), Widowed (W, red), Divorced (D, green), Unmarried (U, purple) or were left unanswered (BL, light blue).

Patients' answers to Q1 and their corresponding employment status and marital status are displayed in Figure 17. From this data it seems that patients were supportive of establishing a public SCB regardless of whether they were married, unmarried, employed or unemployed (**Note*: Blank data (unanswered Q1) is removed and the remaining data is corrected to display percentage support proportional to the number of patients per category; i.e. 68 patients were

employed, with 57 of them supportive (Q1), thus 84% of employed patients are supportive of public UCB SC banking).

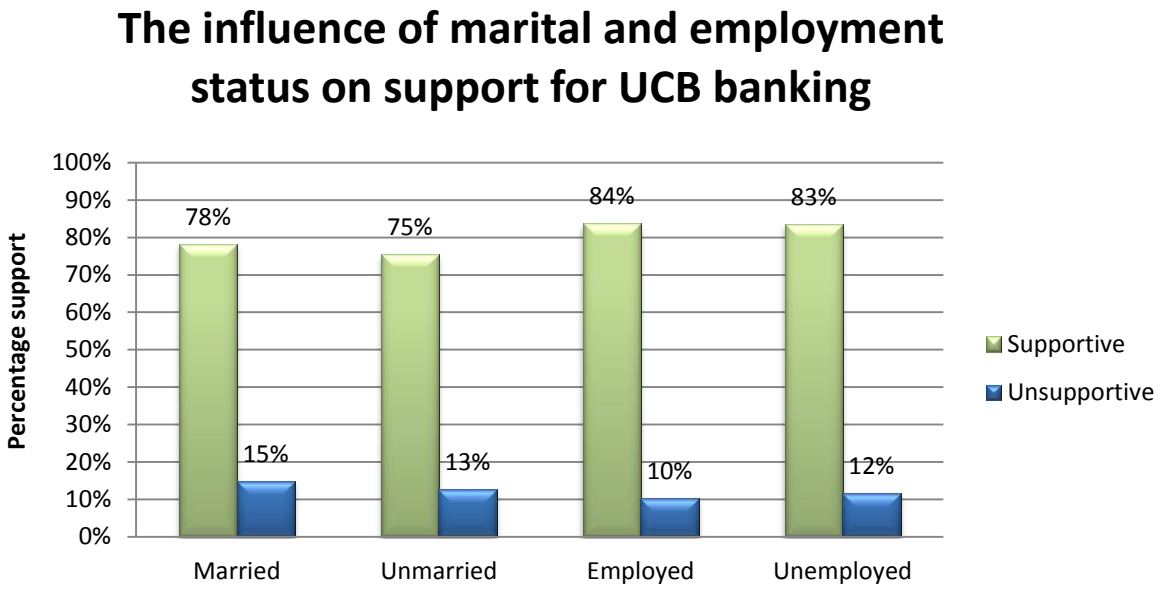

Figure 17: Influence of employment and marital status on patient support for a public UCB SCB (Q1).

From Figure 17 it is clear that marital status and employment status are not determining factors for a patient's support for UCB banking. Equal support was given from married (78%) and unmarried (75%) patients; 84% of employed people supported the bank and 83% of unemployed people supported the bank. A similar trend is observed for unsupportive patients in each of the abovementioned categories.

These results could be because very little is required of patient in order to donate their placentas to UCB collection. If they are presented with adequate information, they would not have a need to contact family or relatives for more information or support in making the decision to donate. Furthermore, eligibility for donation does not require any financial contribution nor does a patient have any additional expenses associated with donation, since the placenta and UCB is collected at the time of delivery of the child.

5.6 Conclusion

South Africa is in a favourable position to implement new avenues for access to healthcare and to increase development in the areas of cellular, molecular and regenerative medicine. Not only are many South Africans in need of these advanced medical and technological developments, but they are also enthusiastic about building a better South Africa.

Similar studies to the one presented here have been conducted globally. Results from this study are strikingly similar to results obtained from these studies abroad and are discussed below:

Fernandez *et al*. (2003) assessed the knowledge and attitudes of Canadian women with regard to testing, collection and banking of UCB SCs. Rucinski *et al*. (2010) reported on the opinions and beliefs of Hispanic and non-Hispanic woman with regards to UCB donation and banking.

Both studies encountered a large gap in information available and accessible to patients, with very few patients aware of UCB banking. As many as 70% of patients indicated poor or very poor knowledge of UCB SCs (Fernandez et al., 2003), which corresponds to data gathered for this study (Fig. 7, Q4) while Katz *et al*. (2010) reported that 79% of woman lacked basic knowledge about SCs.

In the current study the investigator concluded that educating the public with regard to UCB banking and the application thereof would be the single most important factor in generating public support for a public UCB bank. Rucinski *et al*. (2010) concluded the same reporting that their biggest barrier to patient support was a lack of basic information available to the public with regard to UCB banking, UCB harvesting and use. They furthermore suggested that patients should not only be informed on the social value of UCB banking but also be informed about the technical aspects involved in banking.

Similar to observations reported in the current study, Fernandez *et al*. (2010) and Katz *et al*. (2011) both reported a majority support for public UCB banking (as opposed to private or hybrid banking). In this study it was found that a conservative estimate for support from patients for the public bank lies between 80% and 86% (Fig. 3, Q1 and Q6). Taking only workable data into account, these numbers increase to between 85% and 94% (Fig. 7, Q1 and Q6).

Similarly, Fernandez *et al*. (2003) reported that 86% of their Canadian patients opted to store their UCB in a public bank and Katz *et al*. (2011) reported 89% of patients (from 5 European countries) would store their UCB, 76% of which would store publicly.

Additional factors assessed by previously published studies that are similar to results from this study:

Katz *et al*. (2011) found no correlation between patient income and the decision to donate UCB, which held true for all five countries surveyed. Although information gathered about patient employment in the current study is insufficient to draw definitive conclusions, preliminary data seem to correspond with results obtained for patient income from Katz *et al*. (2011).

Many studies have reported on ambiguity of words that caused confusion amongst the patients – most notably "donation" versus "banking", "cord" as explained in "cord blood" (as opposed to spinal cord) (Rucinski et al., 2010). Similarly, this study found words such as "placenta" and "bank" often confused patients. In order to clarify the concepts, words were either translated (e.g. placenta translates to "Inghubo" in Zulu) or explained in broader detail.

Rucinski *et al*. (2010) mentioned that racial and ethnic disparities were observed for donation of UCB similar to those found in organ and tissue donation. Although this does not seem to be the case in South Africa, the possibility that certain ethnic groups in South Africa might be more reluctant to donate cannot conclusively be ruled out in the current study. In order to address potential ethnic influences with regard to UCB banking, the patient cohort would need to be more representative of the population. The questionnaire should also be modified to capture patient ethnicity more accurately.

Rucinski *et al*. (2010) also reported on misconceptions with regards to the placenta, its function during pregnancy and what happens to it after pregnancy. This is similar to observations made during the pilot study (reported on earlier) accounting for the lack of patient knowledge of even basic biological concepts.

This study was conducted to estimate public preparedness and support for establishing a public UCB SCB in South Africa. It furthermore obtained information on specific parameters that could potentially impede the establishment of such a bank. Although the patient cohort was not adequately representative of the total population of South Africa, patients were nevertheless optimistic about the potential establishment of a public bank. Comments received from

patients during the study were predominantly positive, stating their enthusiasm and support for an UCB bank. Reasons from unsupportive patients were mostly reflective of unavailable and inadequate information available to the public with regard to UCB donation.

Patients were supportive of UCB banking regardless of their age, ethnicity, employment, marital status or whether they had previously experienced childbirth. Some of these factors – e.g. previous childbirth – might, however, influence the level of a patient's understanding of UCB donation.

The main determining factor in obtaining support for UCB banking in SA is thus equipping the patients with adequate and accessible information in order to make a properly informed decision. This information should be tailored to address not only people with different levels of education but should also be 'age friendly'; i.e. should accommodate older people's disadvantage with regard to technological development and comprehension. The information should include technical aspects involved in the processes of UCB donation, banking and application as well as the social value of donation.

Finally, results obtained from this study are supportive of establishing a public UCB SCB in South Africa but should be confirmed in different provinces across the country. It serves as a preliminary screening of the public acceptability response from a selected cohort of South African citizens to UCB public banking and paves the way to an in-depth social scientific enquiry. These results could potentially also allude to provinces that might be more suitable for the establishment of public UCB banks.

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CHAPTER 6

6 Verification of the Ultrio-Plus® assay on umbilical cord blood

6.1 Introduction

Umbilical cord blood (UCB) has become an acceptable alternative source of haematopoietic stem cells (HSCs) for bone marrow (BM) transplantation (Broxmeyer et al., 1990; Brunstein et al., 2007). The main function of a public UCB stem cell bank (SCB) is to collect and appropriately store voluntarily donated UCB, until such a time that any patient might need the UCB unit for transplantation. UCB units collected for a public UCB bank would thus be for allogeneic purposes.

With South Africa's particularly high rate of HIV infections, one of the biggest challenges in the establishment of a South African public UCB bank is to screen effectively for infectious diseases and in particular for HIV prior to storage of a unit. Current international screening methods involve screening of the donor (mother) for infectious diseases and potential risk factors associated with rejection of a donated UCB unit. No tests have thus far been verified to screen the UCB unit itself for infectious diseases.

6.1.1 Occurrence of HIV-1 infection in South Africa

South Africa is faced with enormous challenges in the areas of HIV prevention (including education) and treatment. With regard to the prevalence of HIV/AIDS in South Africa, the data from the South African Antenatal Sentinel HIV and Syphilis Prevalence Survey reveal the following (Anon, 2010a):

- The estimated overall HIV prevalence rate is approximately 17.9%. The total number of people living with HIV is estimated at approximately 5.57 million. For adults aged 15 to 49 years, an estimated 17% of the population is HIV positive (Anon, 2010a).
- For 2010, approximately 4.03 million people aged 15 and older and approximately 438 000 children were infected with HIV (Anon, 2010a).
- Of these individuals, 1.2 million people aged 15 and older and 102 000 children would be in need of anti-retroviral therapy (ART) (Anon, 2010a).
- The total number of new HIV infections for 2010 is estimated at 281 000 for adults, and 54 000 new infections among children 14 years and younger (Anon, 2010a).

Figure 18 below indicates the global prevalence of HIV and puts the severity of HIV prevalence in sub-Saharan Africa into perspective: where most countries have an estimated HIV prevalence below 5%, sub-Saharan Africa has an estimated HIV prevalence of more than 15% to 28%; i.e. three to six times that of most countries in the world.

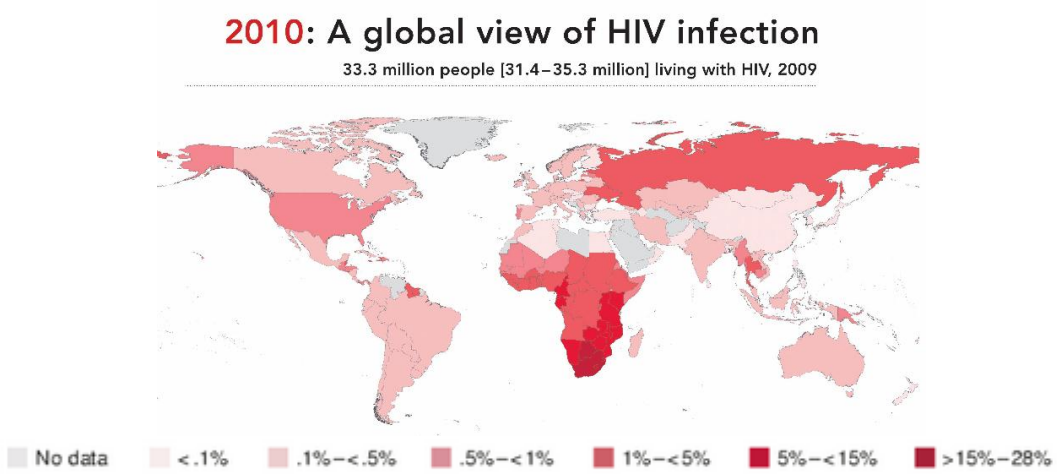

Figure 18: UNAIDS report on the global Aids epidemic, illustrating global HIV prevalence for 2010 (http://www.unaids.org/globalreport/HIV_prevalence_map.htm).

Figure 19 indicates the estimated HIV prevalence in 15 to 49 year olds for individual provinces in the country. It shows four of the nine provinces (Gauteng, Free State, Mpumalanga and KwaZulu-Natal) with HIV prevalence rates above 30% and KwaZulu-Natal almost reaching 40% prevalence.

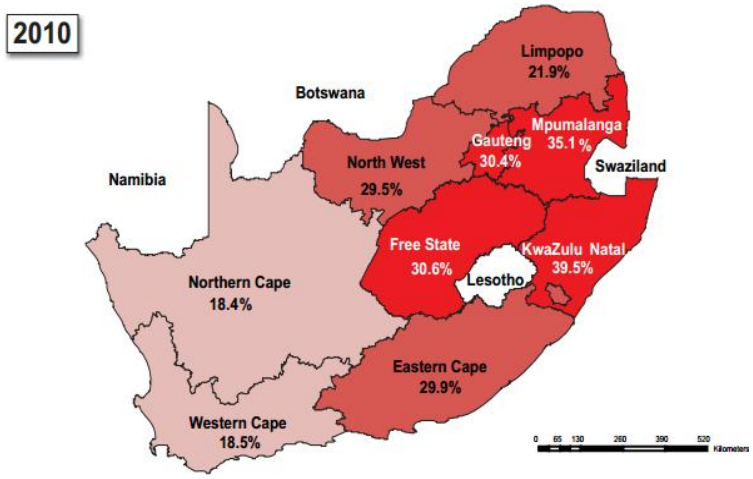

Figure 19: HIV prevalence among antenatal women, distribution by province, South Africa, 2010 (Anon, 2010a)

Of particular importance for establishing a public UCB bank is the high HIV infection rates among pregnant women, from whom UCB units would be obtained. According to this survey, the national HIV prevalence for woman attending antenatal clinics in 2010 was estimated at 30.2% (95% CI of 29.39 to 30.91). The HIV prevalence trend from 1990 to 2010 among women attending antenatal clinics is indicated in Figure 20 while Figure 21 shows the trend in individual provinces in SA from 2008 to 2010.

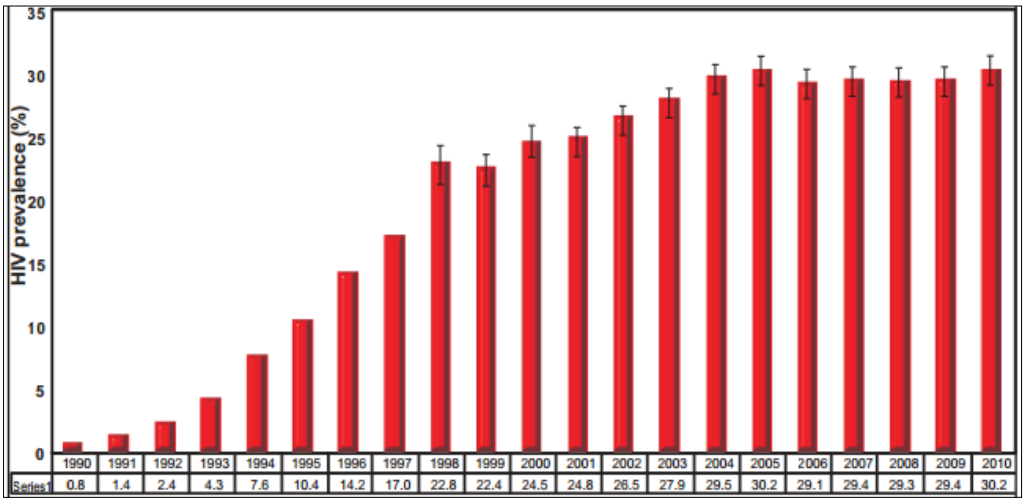

Figure 20: HIV prevalence trends among antenatal women, South Africa 1990 to 2010. The estimates from 2006 are based on a different sample from the previous years (Anon, 2010a)

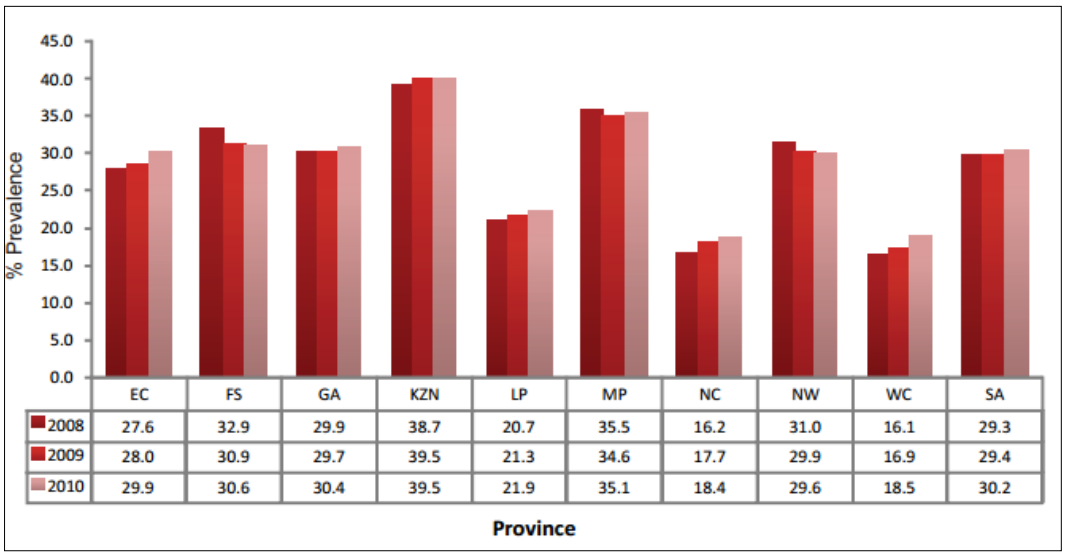

Figure 21: HIV prevalence trends among antenatal women by province, South Africa, 2008 to 2010 (Anon, 2010a)

It seems that the sharp increase in HIV prevalence from the early 1990s has levelled out since 2004 and has remained more or less stable at 29% for the past four years. These high

prevalence rates would disqualify significant numbers of potential UCB units even before collection. This underscores the importance of pre-screening questionnaires for the mothers so that only potentially viable UCB units are collected and unnecessary downstream screening expenditures are prevented.

In addition to the dramatic effects that HIV/AIDS has on individuals, families and society in general, an indication of the impact of HIV/AIDS and tuberculosis (>70% of patients with TB have HIV/AIDS) on the South African economy can be found in the Global Competitiveness Index, which is determined by World Economic Forum. In the 2012 to 2013 period, South Africa was ranked 50th overall out of 144 countries (web: http://www3.weforum.org/docs/WEF_GlobalCompetitivenessReport_2012-13.pdf).

However, when health was considered on its own, the following ranking data emerged:

Category	Rank
• Health	131
• Business impact of HIV/AIDS	135
• Tuberculosis incidence	143
• HIV prevalence	141
• Business impact of tuberculosis	132
• Life expectancy	133
• Infant mortality	107
• Business impact of malaria	100
• Malaria incidence	89

This points to the dramatic effect that infectious diseases (including HIV/AIDS) have on South Africa's global competitiveness.

6.1.2 Probability of obtaining HIV-1 positive umbilical cords: vertical transmission of HIV-1 from mother to child

HIV infection and transmission can occur in utero and is termed mother-to-child transmission (MTCT), vertical transmission or trans-placental transmission (Soilleux & Coleman, 2003). In developed countries, the prevalence of HIV-1 MTCT ranges between 13% and 32%, while it increases to between 25% and 48% in developing countries, with 30% of these HIV-positive infants being infected "in utero" (Guevara et al., 2000). A study done by Taha *et al*. (2011) furthermore suggested that the percentage of in utero infection increases with newly infected mothers. These authors found that out of a total of 73 mothers, recently infected mothers

transmitted the virus in utero at a frequency of 17.8%, as opposed to not-recently-infected mothers who had an in-utero transmission of 6.7% (Taha et al., 2011).

Studies that furthermore distinguish between true in-utero infection, intrapartum infection (occurring during the time of birth) and perinatal infection (period around birth – between five months before and one month after birth) are in agreement that around 5% to 8% of HIV MTCT occurs in utero, while 15% to 30% occurs intrapartum (Biggar et al., 1996; Mock et al., 1998).

Guevara (2000) stated that HIV RNA measurements from maternal and cord blood plasma allow for the quantitative assessment of HIV viremia in the mother and infant respectively. This statement was further supported by Biggar *et al*. (2007) who reported that they conducted polymerase chain reactions (PCR) on infants to detect the HIV genome. The infants were only considered to be infected with HIV in utero if HIV was detected by PCR done on umbilical cord blood. They furthermore concluded that the positive infants were indeed infected in utero due to HIV levels equally as high as, or higher than their mother's HIV levels.

Courgnaud *et al*. (1991) reported that they detected HIV DNA (provirus) in foetal spleen thymus and peripheral blood mononuclear cells (PBMCs). The foetuses were aborted between 16 and 24 weeks from HIV-positive mothers. Nine foetal specimens of spleen and PBMC and eight foetal specimens of thymus were tested for HIV DNA. Six out of the eight foetal thymus specimens, eight out of the nine spleen foetal specimens and five out of the nine PMBC foetal specimens tested positive for HIV DNA, demonstrating that HIV infection does occur in utero.

HIV infection of Hofbauer cells, specialised foetal macrophages, has been demonstrated by *in situ* hybridisation, *in situ* PCR and immunohistochemistry (Newell et al., 1998). HIV has also been detected in amniotic fluid (Guevara et al., 2000; Newell et al., 1998).

Townsend *et al.* (2008) found in their study on mothers receiving ARTs, that three infants (from a total of 2117 infants born) contracted HIV from their mothers despite the mothers being on ART treatment and having viral loads below 50 IU/mL. Two of these infants showed evidence of in utero transmission.

6.2 International regulatory standards for screening of UCB units

Upon receiving an UCB unit, the unit has to undergo various types of screenings in order to medically qualify it for transplantation. Each unit receives a "Cord blood unit report", which contains detailed information about the unit – e.g. total nucleated cell (TNC) count, human

leukocyte antigen (HLA) typing, specific tests performed on the CB unit and/or mother to name but a few (Welte et al., 2010).

Cord blood banks have maternal health questionnaires that serve as a pre-screening tool and aim to identify certain risk factors related to transplantation of the UCB unit prior to acceptance or storage. These risk factors vary between different cord blood banks, but the World Marrow Donor Association (WMDA) has consolidated these requirements into a comprehensive list. The list covers various blood disorders (red and white blood cells and platelets), certain genetic disorders (including monogenic disorders), cancers (Leukaemias), metabolic disorders, severe auto immune disorders and infectious diseases (Welte et al., 2010).

There are currently three potential ways of screening for infectious diseases:

1. Screening the mother within seven days of delivery
2. Retesting of maternal donors at six- month follow up
3. Testing the UCB unit

6.2.1 Maternal screening:

As per Section D 11.1.9.2 of the NetCord-Foundation for the Accreditation of Cellular Therapy (FACT) International Standards for Cord Blood Collection, Banking and Release for Administration (fourth edition) (Anon, 2010b2), the minimal evaluation of infectious agents is performed through serologic screening and nucleic acid testing (NAT) of the maternal sample as a substitute for the CB unit.

Pregnant mothers get tested for HIV at their first medical consultation. If they are found to be HIV positive, anti-retroviral regimens are administered to them in order to prevent MTCT of the virus. If a patient is negative at the first screening for HIV during pregnancy, it does not rule out the possibility that she might still contract HIV during her pregnancy. Mothers that consented to UCB donation are therefore screened again for infectious diseases – including HIV – within seven days prior to or after delivery. In order to rule out the possibility that the mother might be in the window period of infection at the time of her last screening, some cord blood banks also require an additional follow-up screening of the mother six months after delivery. In such a case, a CB sample would only be eligible for further consideration if the screening results for all the time periods are negative.

Although there are benefits to conducting a six-month follow-up screening on the mother, it places an administrative burden on the cord blood banks. It is often difficult to locate the patients after six months and many might not stay close to the hospital or clinic. The onus of re-testing the mother lies on the bank and the bank would therefore be responsible for any additional costs involved for the patients to return to the hospital or clinic for screening. The NetCord-FACT guidelines in dealing with cases where six-month follow ups of the mother are not achievable are found in Section B.2.6.6.2. If initial maternal screening results return as indeterminate or repeatedly reactive, the UCB bank cannot conclude on the interpretation of results without a follow up on the mother. Therefore, the NetCord-FACT guidelines suggest that the UCB bank inform the mother and / or physician of the test results in order to rule out potential health-related risks (Anon, 2010b2).

6.2.2 Cord blood unit screening

Another alternative would be to screen the mother at the time of delivery but to also subject the UCB unit to screening. According to Section D. 10.8 of the NetCord-FACT Cord Blood Accreditation Manual, testing of the CB units are recommended. Many test kits (for infectious diseases) have not been approved by the Food and Drug Administration (FDA) for use on UCB, but performing these tests is nevertheless recommended by the NetCord-Foundation. In the case where a screening test – which is unaccredited for UCB – is used, the UCB bank is advised to denote the outcome and annotate that the test has not yet been validated (Anon, 2010b).

Section D 10.8 of the NetCord-Foundation furthermore states: “Prior to the release to the Clinical Program, each Cord blood unit should be tested for evidence of infection by at least the following communicable disease agents using licensed donor screening tests when available according to Applicable Law”:

- Human immunodeficiency virus type 1
- Human immunodeficiency virus type 2
- Hepatitis B virus
- Hepatitis C virus
- Human T cell lymphotropic virus type 1
- Human T cell lymphotropic virus type 2
- Treponema pallidum (syphilis)

- And any additional agents required by Applicable Law at the time of the release of the CB unit" (NetCord FACT international standards) (Anon, 2010b).

The reluctance to standardise screening of UCB units stems from concerns about reducing the volume of the UCB unit for additional testing requirements. Volumes might furthermore be affected by dilutions with the anti-coagulant in the collection bags. Furthermore, if appropriate provision for testing and re-testing were not made, it might require thawing of the UCB unit, which could damage the integrity of the sample (Anon, 2010b). However, in order to overcome this last-mentioned logistical issue, small segments attached to the UCB bag are now being sealed off and frozen together with the CB unit during sample processing. These segments are representative of the UCB unit and can easily be broken off and used for additional screening or sample analyses without compromising the UCB unit's integrity or volume.

It therefore seems that the more viable option, which would also be the most stringent in screening for infectious diseases, would be to screen the UCB units in addition to screening the maternal sample within seven days of delivery.

6.2.3 Stringency in screening and acceptance criteria

The heavy burden of HIV disease in South Africa combined with the risk of MTCT highlight the important risk of obtaining and transplanting potentially infected UCB units. UCB banks make their UCB units available to patients globally: however, these risks might discourage international UCB banks from using UCB units that originate from South African UCB banks.

In order to increase stringency of detection methods for infectious diseases, tests need to be validated/verified for use on UCB units in addition to already validated tests currently performed on peripheral blood. This would increase screening comprehensiveness and improve international confidence in the quality of UCB units.

By only screening the donor (mothers) for infectious diseases, certain HIV-1-infected UCB units could go undetected. It is important to note that although the placenta serves as a barrier to entry to disease organisms, the extent of vertical transmission of diseases varies between different organisms. Conversely, it might be argued that potentially viable UCB units would be wasted if they were discarded only on the basis of the mother's history of infection. Performing screening on both the maternal sample and UCB unit would increase the safety margins and decrease margins of error when screening is performed.

6.2.4 Transplantation of UCB units for HIV-positive patients

The question has arisen whether organ donation and transplantation for HIV-positive individuals might be feasible if done between HIV-positive individuals. Because of the success of ART therapies in reducing viral load, HIV-positive patients live longer, healthier lives than before and subsequently also become subject to other diseases affecting organ function.

Most HIV patients are not eligible for transplantation purposes for numerous reasons – most notably the accompanying suppression of an already weakened immune system in order to prevent graft versus host disease post transplantation. However, studies done on kidney transplantation between HIV-positive individuals have delivered promising results (Muller et al., 2010; Frassetto et al., 2009), making the possibility of eligible HIV transplantation donors and recipients a reality.

Similarly, the question arises as to whether or not HIV-positive patients' UCB units should not be stored for potential use for another HIV-positive individual.

Currently, UCB units are not collected from patients who have received ART treatment during their pregnancies. Although there is a risk of trans-placental transmission of HIV during pregnancy, this risk decreases significantly when mothers receive ART treatment. A study done by Townsend *et al.* (2008) on perinatal transmission of HIV in 5,151 HIV-infected women in the United Kingdom and Ireland between 2000 and 2006 showed transmission rates as low as 1.2% (61/5151, 95% confidence interval: 0.9-1.5%), and 0.8% (40/4864) for women who had received ART for at least the last 14 days of pregnancy (Townsend et al., 2008).

If the viral load of a mother receiving ART is below 50 IU/mL and the subsequent screening of the collected UCB unit is negative, should this CB unit be discarded, made available to the general public (seeing that it is negative) or be stored separately for potential use in HIV-positive patients? These answers would be subject to stringency and sensitivity of tests used and the reliability of results. Many HIV-negative individuals would probably not be comfortable with receiving an UCB unit (albeit negative) from an infected mother regardless of her current health status. In these cases, it might be best to keep these samples separate from samples that were qualified as negative for both maternal and UCB unit screening.

In a country as severely affected by HIV as SA, it might, however, be necessary to create a separate storage facility that would only store UCB units collected from HIV-positive individuals. If both the mother and UCB unit are screened, then samples from HIV-positive patients could

be divided into three categories: A) Screening where both maternal and UCB unit returned positive; B) Maternal sample resulted positive, but the UCB unit came back negative; C) Mother had a history of infectious diseases and/or used ART but current viral load is undetectable and both maternal sample and UCB unit returned negative.

A critical component of clarifying these concerns will be the accuracy and sensitivity of tests used to detect the various infectious diseases.

6.3 Ultrio-Plus® assay

The Ultrio-Plus® assay is a nucleic acid test (NAT) that has been validated for the simultaneous detection of HIV type-1 (HIV-1), Hepatitis B-Virus (HBV) and Hepatitis C-Virus (HCV) in human peripheral blood (PB), bone marrow (BM) and cadaveric tissue (using plasma or serum). The test was developed, manufactured and distributed by Gen-Probe Inc. (San Diego, CA) in collaboration with Novartis Vaccines and Diagnostics, Inc (Emeryville, CA). It utilises target amplification nucleic acid probe technology and has an internal control incorporated for monitoring assay performance in each individual specimen. Although it does not discriminate initially between a positive signal for HIV-1, HBV or HCV, the technique is fast, effective and accurate in determining which samples are contaminated with these infectious diseases and should be discarded. Specimens found to be reactive in the Ultrio-Plus® assay can be run in individual HIV-1, HCV, and/or HBV discriminatory assays to determine if they are reactive for HIV-1, HCV, HBV or any combination of the three, should the need arise.

6.3.1 Procleix® ultrio® assay (Ultrio-Plus® assay)

The following section has been modified from the package insert:

The Ultrio plus assay is used internationally by blood centres (including the South African National Blood Services (SANBS)) for HIV-1, HBV and HCV screening.

It has three main steps:

1. Target capture (sample preparation)
2. Transcription-mediated amplification (TMA)
3. Detection of the amplicon (amplification products) by the hybridization protection assay (HPA)

6.3.1.1 Step 1: Target capture

The aim of the first step is to isolate the target (HIV-1 RNA, HCV RNA and HBV DNA). In the case of HIV, this involves the release of viral genomic RNA, the denaturation of proteins and the solubilisation of the viral envelope by adding a detergent to the sample in question. The next step in the target capture is to hybridise oligonucleotides (short nucleic acid polymers) that are homologous to highly conserved regions of HIV-1 to the HIV-1 RNA if it is present. Finally, in order to separate the hybridised HIV-1 RNA, it is captured by magnetic micro-particles, which are separated from the sample in a magnetic field. Subsequent wash steps remove extraneous components from the reaction tube.

6.3.1.2 Step 2: Transcription-mediated amplification

The aim of this step is to amplify the hybridised HIV-1 RNA through a process called "transcription mediated amplification". The hybridised HIV-1 RNA has to be converted into a DNA copy of the target sequence. This process is achieved by the enzyme, reverse transcriptase. In the case of the Ultrio-Plus® assay Moloney Murine Leukaemia Virus Reverse Transcriptase (MMLV reverse transcriptase) is used. The DNA copy contains a promotor sequence for the T7 RNA polymerase enzyme. This enzyme in turn produces multiple RNA copies from the DNA amplicon.

6.3.1.3 Step 3: detection of the amplicon by HPA

Detection of viruses occurs through a process known as "hybridisation protection assay" (HPA). Complementary single-stranded nucleic acid probes with chemiluminescent labels are hybridised to the specific amplicon. A selection reagent is then added. The selection reagent differentiates between hybridised and unhybridised probes and inactivates the probes on the unhybridised single-stranded nucleic acid. The hybridised probes give off a chemiluminescent signal, which is measured by a luminometer and reported as Reactive Light Units (RLU).

The Ultrio-Plus® assay is used to detect HIV-1 RNA, HCV RNA and HBV DNA simultaneously. In order to differentiate between these three viruses the Procleix HIV-1, HCV, and HBV discriminatory assays need to be done. The discriminatory assays follow the same three steps as described above except that they use HIV-1-specific-, HCV-specific-, or HBV-specific probe reagents in place of the Ultrio-Plus® assay Probe Reagent.

The Ultrio-Plus® assay has not been validated for cord blood plasma, which – for all intents and purposes – should be similar to plasma from peripheral blood. Method verification of the Ultrio-Plus® assay done on UCB plasma would be advantageous for screening any UCB

unit intended for UCB banking and subsequent transplantation. Results obtained from maternal screening could then be compared to UCB plasma Ultrio-Plus® results.

6.4 Hypothesis and objective

Should a public UCB bank be established in South Africa, all UCB units would undergo compulsory routine infectious diseases screening for compliance with international regulatory standards. It would be imperative to have a sensitive and reliable assay for detection of HIV-1 in potential UCB units prior to banking. It would also be beneficial to use the same screening test for both maternal samples and UCB units for further result comparison.

Since the Ultrio-Plus® assay has been validated for specificity and sensitivity in PB and BM samples, the investigators hypothesised that it would also be an effective, sensitive assay for successful detection of HIV-1 in UCB units. The objective is thus to verify the routinely used Ultrio-Plus® assay for sensitivity in detection of HIV-1 in UCB units.

6.5 Methodology

The Ultrio-Plus® assay has previously been validated for specificity and sensitivity in peripheral blood samples. The researchers wanted to verify that sensitivity of the assay would not be compromised when UCB plasma was used. UCB units were collected at the Steve Biko Academic Hospital from expectant mothers that had given informed consent to use their UCB for medical research. UCB was collected in UCB collection bags (Pall Medical, Midrand SA), containing citrate phosphate dextrose (CPD) anticoagulant. Units were plasma depleted during centrifugation (800 rpm) for 20 min. and the plasma stored in accordance with the Ultrio-Plus® assay protocol for human serum or plasma according to the package insert guidelines, until further sample processing could commence. Because of difficulties of obtaining HIV-positive UCB units for screening purposes from mothers that were already receiving ART, the researchers decided on spiking 16 UCB units with HIV-1 with a known viral load for validation purposes.

According to the Ultrio-Plus® protocol, whole blood, plasma, or serum may be stored at temperatures ≤ 25°C for up to 72 hours from the time of withdrawal, temperatures that exceed 30°C are acceptable for no more than 24 hours. Specimens may be stored an additional five days at 2° to 8°C following centrifugation. Plasma separated from the cells may be stored for longer periods of time at ≤ -20°C before testing. For validation purposes, collected UCB units

were stored at 2° to 4°C for less than 72 hours, after which they were plasma-depleted and plasma was stored at -20°C until the Ultrio-Plus® assay could be performed on these samples.

In order to obtain a panel of ten UCB units for validation purposes, 16 UCB plasma units were run in the Ultrio-Plus® assay to assess HIV-1 sensitivity (i.e. IUmL). The 16 UCB units were screened by the Ultrio-Plus® assay prior to spiking them with HIV-1, in order to confirm their HIV negative status. UCB samples were subsequently spiked with three dilutions (1:2, 1:4 and 1:8) of an HIV-1 positive quality control stock (diluted 1:80) with a known HIV viral load (VL) (used by the SANBS). The viral loads added to the dilutions were thus 46 IUmL (1:2 dilution); 23 IUmL (1:4 dilution); and 11,5 IUmL (1:8 dilution) respectively. All samples were run by the SANBS through the Ultrio-Plus® assay according to already existing protocols in order to verify the Ultrio-Plus® assay's sensitivity for detection of HIV in UCB plasma. Figure 22 gives a diagrammatic representation of the dilution procedure.

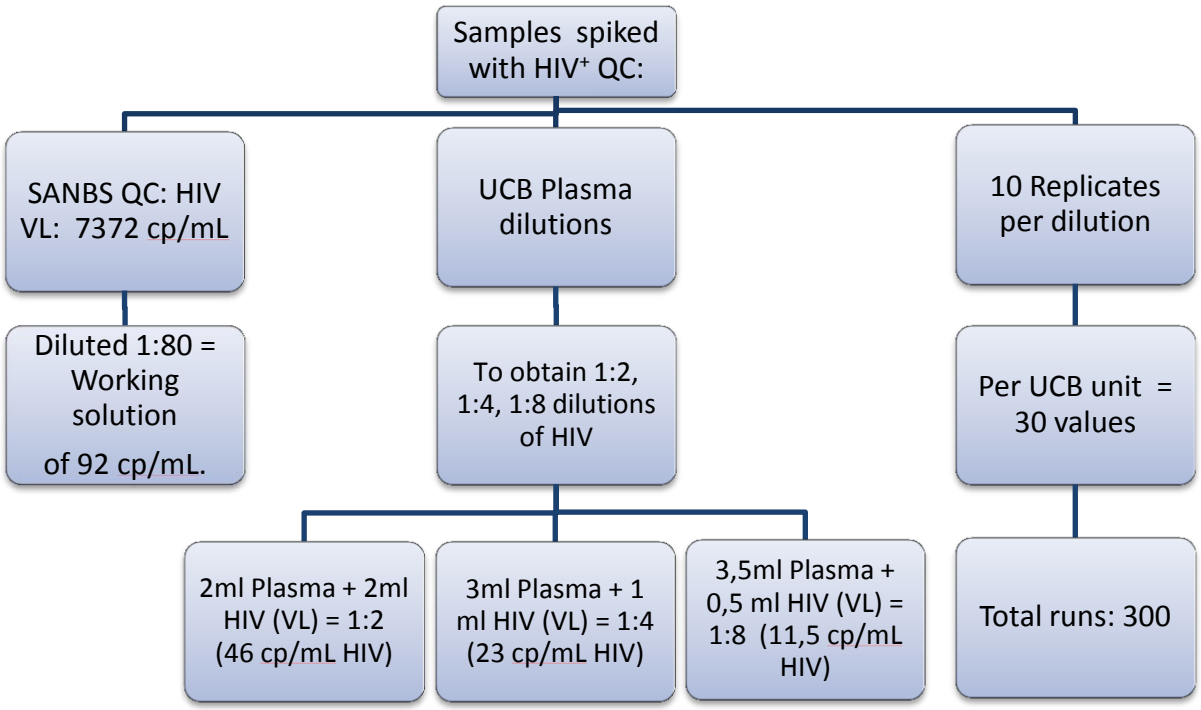

Figure 22: Procedure followed for the 10 UCB units used during validation of the Ultrio-Plus® for sensitivity. A known HIV VL QC stock solution was diluted (1:80) and used as working solution for further 1:2, 1:4 and 1:8 times dilutions. Each sample had three dilutions, which were repeated 10 times each for a total of 30 values per patient and 300 values in total.

The test was compiled so as to prove reproducibility of sensitivity of the assay on UCB units up to the lower detection limit as is currently accepted for screening of PB samples.

Samples were run through the Ultrio-Plus® assay and results documented in Microsoft Excel (Microsoft Corp., Redmond, WA). Commercially available quality control kits, as well as internally manufactured quality control (QC) samples (specific to the South African genotypes), prepared by SANBS were used. All quality control procedures, standards and acceptance criteria, as indicated on the Ultrio-Plus® assay insert, were followed for validation of UCB plasma samples.

6.6 Results and discussion

6.6.1 Introduction

Sensitivity and specificity for the Ultrio-Plus® assay have previously been determined and information can be obtained from the package insert. Specificity for the following genetic variants were previously obtained for both the Ultrio-Plus® assay, as well as its subsequent discriminatory assay: HIV-1 specimens and tissue culture isolates of group M (subtypes A, B, C, D, E, F, and G), N and O. Table 3 is taken from the package insert and indicates the specificity of the Ultrio-Assay test for HIV genetic variants.

Table 3: Procleix® System detection of HIV-1 genetic variants with the Procleix® Ultrio® and HIV-1 Discriminatory Assay

Genetic Variant	Conc. IUmL	Ultrio	dHIV-1
HIV-1 Group M Subtype A	300	7/7	7/7
	100	7/7	7/7
	30	7/7	7/7
HIV-1 Group M Subtype B	300	5/5	7/7
	100	5/5	7/7
	30	4/5	7/7
HIV-1 Group M Subtype C	300	8/8	8/8
	100	7/8	8/8
	30	5/8	8/8
`HIV-1 Group M Subtype D	300	7/7	7/7
	100	7/7	6/7
	30	7/7	7/7
HIV-1 Group M Subtype E	300	6/6	7/7
	100	6/6	7/7
	30	6/6	7/7
HIV-1 Group M Subtype F	300	4/4	6/6
	100	4/4	6/6
	30	4/4	6/6
HIV-1 Group M Subtype G	300	2/2	3/3
	100	2/2	3/3
	30	2/2	3/3
HIV-1 Group N	300	1/1	1/1
	100	1/1	1/1
	30	0/1	1/1
HIV-1 Group O	300	7/7	7/7
	100	7/7	7/7
	30	7/7	7/7
HIV-1 Variants Total	300	47/47	53/53
	100	46/47	52/53
	30	42/47	53/53

Samples were not analysed for specificity again, but only for sensitivity.

6.6.2 Sensitivity

According to the package insert, the Ultrio-Plus® sensitivity for running neat HIV-1 specimens is 99.50% with a 95% confidence interval of (CI 98.21 ; 99.94). Diluted specimens (1:8 and 1:16) are given as 98.50% (95% CI 96.76; 99.45) and 98.25% (95% CI 96.43; 99.29) respectively. Table 4 is taken from the package insert and illustrates the analytical sensitivity of the Ultrio-Plus® assay (without looking at the subsequent discriminatory assay data).

Table 4: Procleix® system - Detection of HIV-1 Type B in analytical sensitivity panels

HIV-1 B IU/mL	Number of reactive/ tested^	% Positive	95% Confidence Limits	
			Lower	Upper
300	80/80	100	95	100
100	80/80	100	95	100
30	77/79^	97	91	100
10	55/79^	70	58	79
3	24/80	30	20	41
0	0/79^	0	0	4

^Invalid reactions were not included

According to these results, the Ultrio-Plus® assay detects HIV-1B with 97% accuracy for 30 or more IU/mL. The validation test's HIV viral load dilutions were undertaken in order to go below this copy number, for detection of HIV-1 at 11.5 IU/mL.

6.6.3 Validation results

The average UCB blood volumes obtained prior to plasma depletion varied between 50 ml and 80 ml. In order to perform adequate repeats for each dilution, at least 8,5 ml of UCB plasma was needed per sample. Of the 16 collected samples, only nine UCB units delivered adequate volumes of UCB plasma required for the sensitivity analyses of the assay on UCB plasma. A tenth sample had adequate volume to perform 25 of the 30 repeats and is included in the results displayed in Table 5.

Table 5: Summary of Ultrio-Plus® screening results for 10 HIV spiked UCB units

Patient no	Number of reactive tests per dilution			
	01:02 46 IU/mL	01:04 23 IU/mL	01:08 11.5 IU/mL	Total
1	10	5^	10	25
2	10	10	10	30
3	10	10	10	30
4	10	10	10	30
5	10	10	10	30
6	10	10	10	30
7	10	9^^	10	29
8	10	10	10	30
9	10	10	10	30
10	10	10	10	30

^ *= Invalid reactions due to inadequate sample volume*
^^ *= Invalid analyses due to sample error code related to instrument mechanics*

Each of the ten samples thus had a total reactive score out of 30. For the total of 300 patient samples run, 294 were reactive. Five samples from patient 1 (for the 1:4 dilution) could not be run due to inadequate sample volume while one sample for patient 7 had a mechanical error. If these six samples are not taken into consideration, the test had 100% detection of HIV-1 up to a lower viral load limit of 11 IUmL. Although the viral loads were below those used for initial validation of the test, the results compare well with detection rates observed in Table 4.

6.7 Conclusion

All UCB units intended for storage in an UCB bank would need to undergo infectious disease screening for compliance with international regulatory requirements.

The Ultrio-Plus® assay is a nucleic acid test (NAT) that has been validated for the simultaneous detection of HIV type-1 (HIV-1), HBV and HCV in human PB, BM and cadaveric tissue (using plasma or serum). The test has not, until now, been verified on UCB units.

The current accepted detection limit for screening for HIV infection is 50 IUmL. The Ultrio-Plus® however is more sensitive, with a 95% limit of detection of 21 IU/mL. Although the possibility exists for an HIV-positive sample to go undetected (having viral loads below the currently detectable lower limit), the clinical relevance is yet undetermined.

It is important to furthermore consider the concept of a minimum HIV infective dose. The Centers for Disease Control and Prevention (CDC) reports on the effect of ARTs on the risk for HIV infection: patients that adhere to ART are less infectious than patients without ART with very low or undetectable viral loads (Anon, 2009). A report by Quinn *et al.* 2000 showed that patients who received ART, but still transmitted the virus to their partners, had higher mean viral loads. One infected partner (who received ART) with a viral load of 90,254 IUmL was able to transmit the virus to his partner. In contrast, no transmission took place between partners where the infected partner had a lower viral load of 38,029 IU/mL. It was furthermore found that no HIV transmission took place if the infected partner's viral load was below 1500 IU/mL (Quinn et al, 2000).

HIV infectability is furthermore subject to many different factors. These factors include infective titre, viral load and injection inoculum volume, area of contact (mucosa, blood, etc.) to name but a few. If the right circumstances prevail, a single virion could cause active HIV infection. The probability of HIV transmission in small blood exposures such as with needlestick injury has been investigated by Reid and Juma (2009). They concluded that HIV's 50% infective dose could range from one virion (i.e. two RNA copies) to 65 000 copies.

Until more comprehensive and sensitive methods are developed to eliminate non-detection of HIV-1 positive samples, screening of maternal and UCB units with the Ultrio-Plus® assay is recommended.

According to currently accepted standards and practices, the Ultrio-Plus® assay is as sensitive in detecting HIV-1 in UCB as it is for detecting HIV-1 in peripheral blood. The assay had 100% detection of samples up to a lower detection limit of 11,5 IU/mL and is recommended for future screening of UCB units.

6.8 References

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