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The Belfast Cord Blood Bank

C Bharucha, S Elliott, D Campbell, R Hunter, L McComb

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SUMMARY

The first cord blood bank in the British Isles was established in Belfast in June 1993. Cord blood (CB) is rich in haematopoietic progenitor cells and has been used successfully as a substitute for bone marrow transplants in over 200 patients world-wide. Most have received CB from a histocompatible sibling, but reports include several unrelated HLA matched transplants. In addition to the cryopreservation of 400 units of donated CB in the Cord Blood Bank, we have stored eight CB collections from siblings of children with leukaemia in Northern Ireland. A pilot study in collaboration with the maternity unit in the Mater Infirmorum Hospital confirmed the feasibility of a CB banking programme and highlighted many issues relating to Good Manufacturing Practice (GMP). The authors describe experience of collecting 824 units of CB over three years and discuss a few of the wider implications of this innovation in the management of patients requiring myeloablative therapy.

INTRODUCTION

Umbilical Cord Blood (CB) is a source of haematopoietic stem cells which can be used as an alternative to bone marrow for transplantation.[1] Since the first report of a successful CB transplant[2] more than 200 children and adults with congenital or malignant diseases have received histocompatible CB from a sibling or stored unrelated HLA-matched CB. The Belfast Cord Blood Bank is based within the Northern Ireland Blood Transfusion Service (NIBTS) and consists of dedicated sibling donations for specified patients and unrelated donations for general use.

SIBLING DONATIONS

There is general agreement among haematologists and oncologists that cord blood from a sibling must be cryopreserved if an older child in the family is known to suffer from a disease which is normally treated with bone marrow transplantation.[3] Many CB transplants carried out to date have used sibling collections. We have received requests from the paediatric haematologist for collection of CB from siblings of nine patients with leukaemia when their mothers have become pregnant. Such sibling collections can be arranged in any hospital where delivery is scheduled. The obstetrician in charge is contacted and at least two midwives are trained in the collection procedure, with emphasis on the importance of sterile techniques and accurate documentation. To date, nine sibling collections have been cryopreserved from eight pregnancies (one mother had twins). So far it has not been deemed clinically necessary to use any of the stored units for transplant.

UNRELATED DONATIONS: INFORMED CONSENT

Information is provided to pregnant women by midwives and obstetricians towards the end of pregnancy. In consenting to CB banking, the mother agrees that the collection will not be reserved for any member of her family. At antenatal clinics, mothers are given a leaflet which explains the Cord Blood Bank and the use of CB as an alternative to donated bone marrow in medical treatment. The obstetrician or midwife discusses the programme with the mother,

Northern Ireland Blood Transfusion Service, Lisburn Road, Belfast BT9 7TS.

Chitra Bharucha, MB, BS, FRCPath, Deputy Director NIBTS.

Sarah Elliott, BSc, PhD, Research Scientist.

Doris Campbell, MSc, FIBMS, Medical Laboratory Scientific Officer.

Rosemary Hunter, BSc, Research Scientist.

Lesley McComb, Medical Laboratory Scientific Officer.

Correspondence to Dr C Bharucha.

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answering any queries. The leaflet contains a consent form: the mother gives her written consent to the collection of CB post-partum and consents to provide blood samples for all mandatory tests. The mother is assured that blood is not taken from the baby.

COLLECTION OF CORD BLOOD

Normal obstetric practice is not altered in any way for purposes of CB collection. The obstetrician is in charge of the collection procedure and the actual collection is performed by midwives with the placenta *in utero*. Midwives are trained by a member of NIBTS staff. After delivery, the cord is clamped and cut, and using appropriate aseptic techniques the umbilical vein is punctured. We use standard blood collection sets incorporating a bag containing anticoagulant, sterile tubing and a needle which is used to pierce the umbilical vein; blood is allowed to drain into the bag under gravity.

In addition to sterile collection of the CB, a small sample is milked from the cord into a tube for use as an archive serum sample. A venous sample is collected from the mother for microbiology tests.

Midwives complete forms to provide the mother's details and those of the birth. Thorough documentation of the CB donation is meticulously carried out during collection and processing of samples. Unique barcode labels are affixed to all samples and to the CB pack. These labels are specific for each collection and are an integral part of the documentation system for the CB donations. Any labels that remain unused are returned to NIBTS with the collection.

PROCESSING OF CORD BLOOD COLLECTIONS

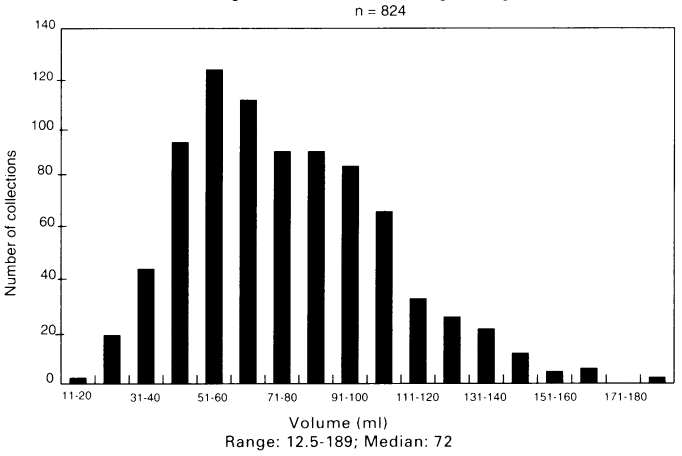
CB is transported each morning from maternity units to NIBTS under the strictly controlled conditions which we use for blood and blood products. At NIBTS all details are recorded and 3 ml CB is removed under sterile conditions from the blood pack using a needle and syringe. This sample is used for ABO and Rhesus grouping, HLA typing, bacterial cultures and nucleated cell counts.

The remaining CB in the pack is mixed with an equal volume of cryodiluent prior to freezing. The cryodiluent consists of sterile dimethyl sulphoxide (DMSO) and albumin in a ratio of 1:4; this protects the integrity of the stem cells. DMSO is exothermic and care is taken to add chilled cryodiluent slowly to CB with continuous mixing. The final mixture for freezing is double the volume of the CB and contains DMSO at a concentration of 10%. The CB/cryodiluent mixture in a freezing bag is placed between metal plates and frozen in a controlled rate freezer to a temperature of -100°C. Subsequently, the frozen packs are transferred to liquid nitrogen tanks where they are stored in the vapour phase.

RESULTS

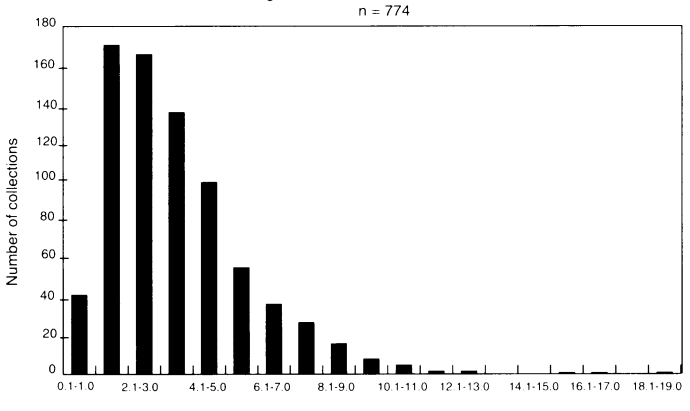
A total of 824 units of CB were collected, of which 186 are available for unrelated transplantation and the remaining collections are used for validation, quality assurance procedures and development of improved techniques for storage. There is a wide range in the volume collected (12.5 to 189ml) with a median of 72ml (Fig. 1). The range in mononuclear cell (MNC) numbers was 0.3 to 18.1 x 10^8/unit with a median MNC of 3.1 x 10^8/unit (Fig. 2). Total nucleated cell numbers were also assessed: the range was 0.1 to 33 x 10^8/unit, with a median of 7.1 x 10^8/unit (Fig. 3).

Figure 1: Volume of CB excluding anticoagulant
n = 824



Range: 12.5-189; Median: 72

Figure 2: Total mononuclear cell count
n = 774



Range: 0.3-18.1; Median: 3.1

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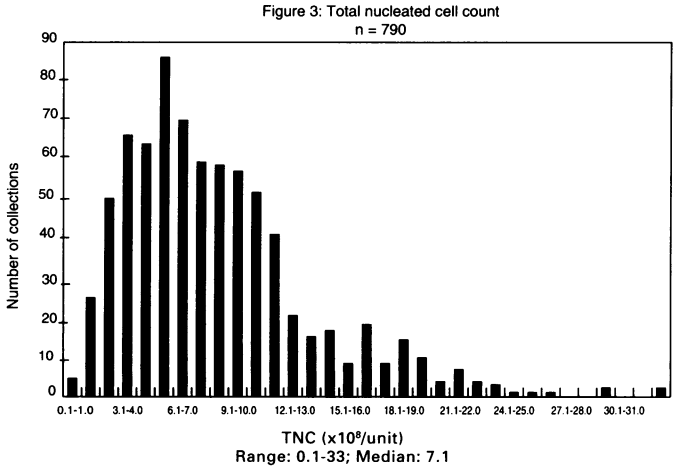

Microbiological screening for mandatory tests include Hepatitis B, Hepatitis C, HIV and syphilis; no positive results were obtained in the 186 collections for which consent for banking was obtained. The bacteriological contamination rate was significant (12.8%) during the early stages of the programme with a brief period when the infection rate was greater than 20%. There was a marked reduction in contamination (4.2%) after midwives were trained in sterile procedures. The organisms cultured were *streptococci, staphylococci* (including coagulase negative strains), *bacteroides, enterococcus faecalis* and *proprionibacterium spp.*

STORAGE AND RETRIEVAL OF CB COLLECTIONS

Documentation is necessary when dealing with blood and blood products and the routine procedures used in NIBTS are extended to CB banking. Identification and accrual of all information relating to tests and subsequent results of HLA typing can be collated for each specimen. Each collection is labelled with a unique barcode number, date of freezing and volume. The special labels must withstand the freezing procedure and are licensed for use in liquid nitrogen tanks. Archive information includes the position of each bag and sample so that rapid and accurate retrieval is assured.

DISCUSSION

CB has a higher concentration of haematopoietic progenitor cells than bone marrow[4]. Furthermore, partially mismatched CB can be used successfully[5] and the incidence of graft-versus-host-disease appears to be infrequent. This is attributed to the immunological naivety of cells in CB. It has been estimated that approximately 40% only of patients who require bone marrow transplantation receive it. Several factors such as a lack of identical HLA matched donors, inability to trace volunteers on the bone marrow donor panel and relapse of disease during the delay in identifying and testing suitable donors have been recognised by clinicians as unsatisfactory[6]. An "off-the-shelf" supply of cryopreserved ("banked") CB will increase the availability of stem cell transplants for patients who require it as part of their management. This is confirmed by the experience in the USA where recent figures indicate that approximately 25% of all unrelated stem cell transplants are from cord blood (Rubinstein, personal communication).

The European Organisation for Cord Blood Banking proposes to collect and store 20,000 CB units in Europe in the next 2-3 years and this will enable us to evaluate the clinical outcome of such a programme[1]. The Belfast Cord Blood Bank is part of this collaborative venture and one of the authors (Chitra Bharucha) is co-ordinator for standardisation of CB banking in Europe. The aim is to store 1000-3000 units of CB in each of the participating centres and to share HLA data with access to compatible units. In the meantime, all the centres are developing techniques for volume reduction of CB for storage without compromising viability of stem cells because of the considerable resource implications associated with liquid nitrogen storage of such a large number of blood bags.

There is no standardisation of processing procedures or tests performed in different centres. Reported results indicate that there is correlation between speed of engraftment and "white cell count" (WBC), "total nucleated cell count" (TNC), "mononuclear cell count" (MNC), CD34 cell count and haemopoietic progenitor colony count. However, these parameters do not provide a uniform accurate determination of stem cell content because of the variation in techniques. Therefore, analyses of clinical results show a wide variation in the optimum CB collection, with one study[5] concluding that a minimum of 1×10^6 TNC/kg is required for engraftment and another study cites 3.7×10^7 nucleated cell dosekg. (Eliane Gluckman, personal communication).

A pilot study initiated in NIBTS in 1993[7] established the feasibility of the collection procedures and banking programme, and highlighted issues of good manufacturing practice (GMP) which have been incorporated into our routine procedures. The wide range in volume

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and mononuclear cells is well recognised[8] and the obstetric factors influencing their recovery have been analysed[9]. The superior engraftment potential and good correlation between volume and MNC concentration make it possible to identify a minimum volume for storage. There are approximately 20,000 deliveries per annum in Northern Ireland. At present, it is necessary to be selective for CB banking and two maternity units have been targeted for training in procedures which comply with GMP requirements. We process and cryopreserve CB collections which are greater than 70ml in volume and have been transported to NIBTS within 48 hours of delivery.

The fact that a significant number of CB stem cells are pluripotent haematopoietic cells makes genetic modification and *ex vivo* expansion possible areas of enormous potential for gene therapy in the future. Commercial companies have been quick in recognising future possibilities, and several companies, at least one of them in Ireland, have begun to exploit patients by offering to store CB indefinitely for an annual fee. This is highly undesirable. Furthermore, there is increasing recognition by the Department of Health and Social Services that CB banking must not be allowed to develop in an unplanned and haphazard manner all over the UK. We are hopeful that funding and procedures for planned further development and extension of the Belfast Cord Blood Bank will be implemented soon.

ACKNOWLEDGEMENTS

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