# Umbilical Cord Blood for Transplantation: from Collection Quality to Its Use in Cerebral Palsy

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**Umbilical cord blood (UCB) has become an alternative source for transplantation in children and adults due to its rapid availability, less stringent human leukocyte antigen–match requirements, lower risk of graft-versus-host disease, and lower risk of infectious disease transmission. Good-quality UCB collection is the first step needed for increasing the successful application of UCB. Since the first use of UCB transplantation to treat Fanconi anaemia in 1988, UCB transplantation has been widely used in both paediatric and adult patients. The rich variety of stem cells other than haematopoietic stem cells in UCB allows potential expansion of its application to the treatment of neurological disorders. In this review, strategies for collecting good-quality UCB, and the benefits and disadvantages of *in utero* and *ex utero* UCB collection methods are discussed. This article also summarises recent developments in the use of UCB transplantation in children and adults, as well as laboratory and clinical evidence of the role of UCB in the treatment of cerebral palsy.**
Hong Kong J Gynaecol Obstet Midwifery 2009; 9:43-8

*Keywords: Blood banks; Cerebral Palsy; Cord blood stem cell transplantation; Hematopoietic stem cell transplantation*

## Introduction

Umbilical cord blood (UCB) is recognised as a rich source of haematopoietic stem cells (HSC) and has been used widely for transplantation in recent years. Approximately 10,000 UCB transplantations (UCBT) have been performed worldwide since the first pioneering UCBT was performed to treat a patient with Fanconi anaemia in 1988[1]. Since then the application of UCBT has been expanded to treat patients with haematological malignancies, immune deficiencies, and inherited metabolic disorders[2]. After two decades of laboratory and clinical research into the use of UCBT, it has become clear that UCB is safe and efficient for transplantation.

UCB is a good alternative to unrelated bone marrow transplantation because it has the following features: (i) it is readily available in cryopreserved form and is human leukocyte antigen (HLA)–typed in cord blood banks, (ii) it is a richer source of HSC than bone marrow, (iii) there are no risks involved during UCB collection after birth, (iv) there is a lower risk of infectious disease transmission, (v) there is tolerance of partial HLA matching (4 of 6 HLA-A, -B, and DRB1 antigens) between donor and recipient, (vi) there is a lower incidence of acute graft-versus-host disease despite HLA disparity, (vii) there are comparable clinical outcomes between unrelated UCBT and bone marrow transplantation in children with acute leukaemia[3]. Nevertheless, the major limitation of UCBT

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is the availability of only a fixed single unit of UCB, preventing a second UCBT or additional HSC when there are relapses or graft failures. The cell dose in the UCB unit is one of the major factors affecting UCBT outcomes. A low cell dose in the UCB limits the use of UCBT, particularly in adults and adolescents[4]. Since UCB collection methods are closely associated with the quality of the UCB unit, it is therefore necessary to improve collection methods.

The emerging field of regenerative medicine has seen dramatic progress as research into stem cells has advanced. One major hope is to make use of the characteristics of stem cells, i.e. their ability to differentiate into different types of cells, to replace defective or damaged tissues. Multiple stem cells other than HSC have been found and isolated in UCB and have shown the ability to regenerate different tissue types. A number of studies have been conducted in sheep and rats using human UCB cells[5,6] and the promising evidence emerging from such studies prompts the exploration of its therapeutic potential for incurable diseases.

This review discusses methods for collecting good-quality UCB, the clinical application of UCBT in children and adults, and a significant clinical trial involving treatment of cerebral palsy (CP) with UCB cells.

## Umbilical Cord Blood Collection: *In Utero* or *Ex Utero*?

A good UCB collection strategy is always the first step for attaining good-quality UCB. There are two distinct collection methods for UCB: *in utero* and *ex utero* UCB collection. *In utero* UCB collection is the collection of UCB from the umbilical vein after delivery while the placenta remains in the uterus[7]. *Ex utero* UCB collection is the collection of UCB after the placenta has been delivered[8]. *In utero* UCB collection is usually performed by midwives or obstetricians in the delivery room, while *ex utero* UCB collection is performed by trained personnel, mainly for UCB collection training purposes, in an adjacent room. Solves et al[9] concluded that the *in utero* UCB collection method is the best approach, allowing optimal UCB banking methodology. Nevertheless, another study conducted by Lasky et al[10] showed that the *in utero* UCB collection method produces a lower volume of UCB, higher bacterial contamination, and UCB clotting. To resolve this conflict, our cord blood bank analysed 2331 units of UCB collected between January 2007 and December 2008 using both *in utero* and *ex utero* collection methods. The preliminary results showed that *in utero* collection yielded significantly higher UCB volumes than *ex utero* collection ($p<0.01$, unpublished data), which concurs with previous studies[11,12]. A higher volume of UCB is closely associated with a higher cell dose, including total nucleated cells (TNC), mononuclear nucleated cells, CD34+ HSC, and colony forming units, all of which are critical factors for successful UCBT[11,13,14]. A possible explanation for the higher UCB volume yielded by *in utero* collection is that the compressing force exerted on the placenta by the uterus expels more UCB. Collection time is also an important factor since blood in the placenta and umbilical cord clots rapidly. Collection time for *ex utero* UCB collection is generally longer and this is directly associated with a higher chance of forming blood clots in placental vessels, resulting in a reduction of UCB stem cells[13]. It is critical that an *ex utero* UCB collection be performed within 10 minutes of placental delivery. The loss of UCB stem cells during an *ex utero* collection may also be explained by the presence of haemorrhage in the maternal and fetal areas of the placenta.

Previous studies comparing *in utero* and *ex utero* UCB collections[9] have yielded inconclusive evidence concerning bacterial contamination[10]. Higher levels of bacterial contamination were found in samples collected *in utero* for unknown reasons. However, *ex utero* UCB collections may also lead to higher rates of contamination due to placental manipulation in an open area after delivery. Although there is no conclusive evidence proving which collection method best minimises the rate of bacterial contamination, use of an aseptic UCB collection technique is always the best practice for preventing bacterial contamination. The standard aseptic UCB collection technique is to first scrub the umbilical cord with alcohol and 10% povidone-iodine swab sticks. This is followed by blood collection from one of the placental veins using a closed bag system in which a needle is connected to a blood collection bag containing anticoagulant. The blood collection bag should be agitated during collection to ensure mixing of the UCB with anticoagulant to avoid blood clotting. Finally, it is critical to seal or clamp the tubing, followed by removal

of the needle, in order to maintain a closed bag system.

## Umbilical Cord Blood Transplantation in Children and Adults

Good clinical outcomes support the use of UCB as an alternative treatment for advanced and high-risk haematological diseases[1,4,15,16]. UCBT has been more successful in children than adults, mainly due to the limitations imposed by the TNC count[17]. The TNC count is a critical determinant that can significantly influence the rate and incidence of haematopoietic recovery in successful UCBT. A limited TNC count in a single UCB unit is usually enough for a child but may not be sufficient for an adult. Matching HLA types is critical for successful engraftment in unrelated BMT, however, a graft with up to two HLA mismatches can result in a higher engraftment rate after UCBT[18,19]. Six out of six HLA-matched UCBT demonstrated 100% improved engraftment when compared with 70-78% in one-to-two out of six HLA-mismatched UCBT although there was no difference between any of the HLA mismatches[18]. A recent study found that unrelated UCBT in children with acute leukaemia had clinical outcomes comparable with unrelated bone marrow transplantation. For this reason UCB is now often the preferred alternative HSC source for children[20].

The encouraging results from use of UCBT in children have prompted more investigations into UCBT in adults. Studies have found no difference in clinical outcomes between the use of UCBT and unrelated bone marrow transplantation in adult patients with acute leukaemia[21,22]. Nonetheless, progress with UCBT in adults remains slow due to the cell dose limitation in single UCB units. Several UCBT approaches have been evaluated to circumvent this limitation including (i) transplantation of *ex vivo* expanded UCB[23], (ii) direct intrabone transplantation of unrelated UCB[24], and (iii) double UCBT after myeloablative therapy[17,25,26]. A minimum of two UCB units both with four out of six HLA-matches (the two do not have to match at the same loci) are needed for a successful double UCBT[25,26]. Of note, patients with acute leukaemia in their first or second remission had a lower incidence of relapse when given double UCBT than those given a single UCBT[27]. An assessment of more than 200 double UCBT showed that double UCBT with two partially HLA-matched UCB units is safe and efficacious, which makes more than 90% of adults eligible for UCBT[26,28]. To date, a single UCBT is generally recommended when a single four-to-six out of six HLA-matched UCB unit with suitable TNC count is available, otherwise a double UCBT is considered the preferred method[29].

## Potential Treatment for Cerebral Palsy

CP encompasses a heterogeneous group of non-progressive and non-contagious motor impairment disorders that arise in the early stages of development[30]. In addition to motor impairment, children with CP develop multiple disabling deficits including mental retardation, epilepsy, visual and hearing impairment, speech and language disorders, and oral-motor dysfunction[31]. CP occurs in about 2 to 2.5 per 1000 births and the prevalence in Hong Kong has been reported to be lower (1.3 per 1000 children) which may be attributable to differences in study design[32,33]. One of the major causes of CP is damage to the developing brain during pregnancy (75%), childbirth (5%), and after birth (15%) resulting in cerebral ischaemic insults and haemorrhages[34]. CP is a lifelong disorder that basically has no cure. CP sufferers tend to be managed with palliative therapies, which include a wide range of medical and rehabilitation services, rather than restorative therapies. Available interventions, such as medication, surgery, equipment, and assistive technology can barely ameliorate CP.

Stem cell properties, particularly their pluri-potency, which allows differentiation into most types of cells, give them promise as a means of replacing or regenerating damaged tissues. The presence of a mixture of different types of stem cells other than HSC, including embryonic-like stem cells, endothelial stem cells, epithelial stem cells, and mesenchymal stem cells in UCB makes UCB stem cells the best alternative to embryonic stem cells. Extensive *in vitro* laboratory studies, animal model studies, and clinical trials in humans have shown that UCB stem cells have potential as treatments for brain injuries and neurological disorders including CP. In one animal study, intravenous administration of UCB into a rat with a traumatic brain injury demonstrated that UCB cells homed in on the injured brain region[35]. Another study showed that intraperitoneal administration of human UCB into a 24-hour post-diagnosis CP rat model resulted in reduced spastic paresis with significant

improvement in walking[36]. The incorporation of UCB cells into the brain lesion suggested that specific chemoattractants, possibly cytokines, released from the damaged region, attracted the migration of UCB cells[37]. The optimal time for transplantation is within the first 2 weeks of the damage. This may be because the chemoattractants might be released soon after the damage has occurred[38]. This 'homing' effect may also be facilitated by the malfunction of the blood-brain barrier in the damaged brain, allowing penetration of UCB cells to the damaged areas[39,40].

Encouraging evidence gained from CP animal studies is now being translated into clinical applications. Autologous UCB infusions given by simple intravenous administration have been performed in a clinical trial on patients with CP. Fifteen patients with CP have been treated in a clinical trial carried out at Duke University (Durham, USA) using their own banked UCB units. The procedure is very simple, involving the intravenous administration of thawed autologous UCB units over 10 minutes. A dramatic improvement in the speech and motor abilities of children with CP was observed after a few weeks of autologous UCB infusion[41]. The only side-effects that have been observed so far were short-term reactions, such as nausea and vomiting, after administration of the autologous UCB together with the freezing medium (such as DMSO) [personal communication, Asia Pacific Cord Blood Bank Consortium, Japan, November 2008]. The frequency and severity of the side-effects were associated with the amount of infused freezing medium. In fact, the concentration of DMSO freezing medium used in UCB cryopreservation is low, and it will be rapidly expelled via exhalation or metabolism after administration. Furthermore, a US Food and Drug Administration–approved clinical trial using autologous UCB infusion for children with traumatic brain injuries is also being conducted at the University of Texas in Houston, USA (personal communication, UT Health Sciences Center, Houston, Texas 2008).

## Conclusion

The variety of stem cells present in UCB enable physicians to not only treat more than 70 life-threatening diseases such as acute leukaemia, aplastic anaemia, multiple sclerosis, and osteopetrosis in children and adults, but also provides hope to patients suffering from chronic incurable diseases. Animal model studies using human UCB to treat neurological disorders such as stroke[42-45], spinal cord injury[46,47], Parkinson's disease[48], and Alzheimer's disease[49] show promise. Furthermore, successful human clinical trials using autologous UCB stem cells to treat CP as well as type I diabetes[50] hold promise for future restorative, rather than palliative, therapies for these patients. It is important to note that each individual gets only one chance in a lifetime to save UCB. Thus collecting good-quality UCB is the key to providing a future lifesaving opportunity. The current and future applications of UCB have led to the establishment of public or private cord blood banks worldwide. It is important for these cord blood banks to be accredited by the American Association of Blood Banks (AABB) or the Foundation for Accreditation of Cellular Therapy (FACT-NETCORD), which provide standardisation of collection, processing, storage, documentation, labelling, equipment control, and cord blood bank operations.

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