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**Transfusion potentialities of Cord blood to combat anemia of any aetiology**

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Cord blood transfusion a new true :Blood Substitute

In a report of the World Health Organization, it was revealed that there are about 500,000 pregnancy related deaths globally, of which at least 25 percent maternal deaths are due to the loss of blood. (1). An estimated 13 million units of blood worldwide are not tested against human immunodeficiency viruses or hepatitis viruses, and in some developing countries 80 percent of the blood supply comes from paid donors or
replacement donors (family friends or acquaintances) even when the infected
population is high. (2).

One of the most important advances in surgery has been the availability of blood and other blood
products. Without the ability to safely give blood during many of the complex surgical procedures that
have saved countless lives, these procedures would not have succeeded. For the last 70 years since
the publication of the report of Amberson (3), there have been global attempts
to find a genuine blood substitute.

Rationality of the use of cord blood as blood substitute:

Aseptically collected placental umbilical cord blood has the richest source of fetal hemoglobin. Fetal
hemoglobin is a natural stress response to hemoglobin synthesis which we try to preserve and augment in
case of thalassemia by providing hydroxyurea or other similar drug supports.

Other conditions like pregnancy, diabetes, thyroid disease, or anti-epileptic drug therapy, can also increase the fetal hemoglobin
concentration. This fetal hemoglobin, with its abundant source, i.e., the
placenta (in India alone, there are more than 20 million placentas produced
as afterbirth every year), is actually a cause of environmental pollution in
many parts of the developing world because it attracts natural scavengers
and spreads infection, unless aseptically treated, or incinerated.

My team of doctors has been successfully transfusing this blood as an
alternative emergency source of blood transfusion in the background of
anemia and emaciation of any aetiology, i.e., from surgery to medicine from
HIV, thalassemia to leprosy or from advanced cancer to patients with a
crippling poliomyelitis, etc. since 1999 (4-12).

To combat the emergency requirement of blood in natural or man
made disaster management, i.e., civil or military due to current global waging war
against terrorism, this precious hypoimmune fetal cells (13-16) with altered metabolic profile is a gift
of the nature, entrapped inside the placenta which could be readily available source of blood not
only in the underresourced countries in the world but in case of the genuine
need for blood substitute anywhere in the world at crisis.

What we have done in this direction is the establishment of clinical safety of allogenic cord blood transfusion in
paediatric to geriatric age group (A study ranging from 1999-2006, funded by
the Dept of Science and Technology, Govt of West Bengal, India).

In the animal kingdom swallowing the afterbirth by the mother is a general
norm. Even herbivorous animals swallow the placenta after the birth of their
babies (for example, the cow). But humans do not seem to know how to use
this precious afterbirth, which has protected and nurtured the baby for so
long in the womb.

The placenta may prove to be a non-controversial source of
hematopoietic and mesenchymal stem cells as well as endothelial progenitor
cells. A "cocktail" of these three elements might be used in the future to
treat one of the more than 80 diseases that have responded to stem cell
transplantation, with potential to treat degenerative diseases such as
heart disease, endocrine disorders such as diabetes, and neurodegenerative
diseases such as stroke, Alzheimer's disease, Parkinson's disease and spinal
cord injuries only to name a few.

Of late, since 1989, (17, 18) consciousness is increasing on the use of umbilical cord blood stem
cells as an easily available source of hematopoietic stem cells for bone marrow
transplantation. These fetal stem cells (CD 34) cause less graft vs. host
reactions after transplantation. Recognition of this potentiality in the
scientific world has resulted in the collection and harvesting of these cord
blood stem cells in many laboratories all over the world. But these hematopoietic stem cells constitute only .01 percent of the nucleated cells of the cord blood. The rest, that is, 99.99 percent of the cord blood is wasted. This wasted gift of Mother Nature is rich in fetal hemoglobin, growth factors and other cytokine filled plasma, and is moreover protected in the infection free environment inside the placenta in case of a healthy newborn.

In the global search for a suitable hemoglobin based oxygen carrier from human RBC to bovine RBC, or its chemically or genetically modified form, or even from sea creatures (Arenicola Merina), i.e., sea worm, the hemoglobin has been extracted (19) for its potential human use. Animal hemoglobin can trigger allergic reactions and can even damage the kidneys. Adult hemoglobin consists of 2 alpha and 2 beta polypeptide chains, each bound to a haeme group, capable of binding with one molecule of O2 (1 Gm hemoglobin binds with 1.39 ml of oxygen ). Therefore, 14 gm percent of adult hemoglobin can carry, on an average, 19.46 ml of oxygen. Cord blood at term carries on an average 16.8 Gm percent hemoglobin (20) of which 20 percent belongs to the adult hemoglobin type (3.36 gms) and 80 percent belongs to the fetal hemoglobin type (13.44gms). The concentration of the fetal hemoglobin may increase further depending on fetal stress, maturity and several other feto-maternal factors. Fetal hemoglobin has the potentiality to carry upto 50 percent more oxygen than adult hemoglobin (21) i.e., 1Gm of fetal hemoglobin may carry upto 2.08 ml of oxygen. If we simply calculate theoretically the oxygen carrying potentiality of 100ml of cord blood taking into account of its 80 percent fetal hemoglobin component (2.08 ml O2 carrying capacity per gm of fetal hemoglobin) and 20 percent adult hemoglobin component (1.39ml O2 carrying capacity per gm of adult hemoglobin ), it would be around 32.62 ml of O2 carrying capacity, which is a 67.62 percent additional oxygen capacity of the adult blood (19.46 ml Oxygen/100 ml). There are several factors which modify the O2 binding affinity, which includes,

(a) concentration of hydrogen ion,
(b) carbon dioxide concentration in the blood ,
(c) body temperature,
(d) 2-3 diphosphoglycerate concentration only, to name a few.

Whether fetal hemoglobin rich placental umbilical cord whole blood which has the potentiality to carry more oxygen to the tissue Vol/ Vol than adult blood because of its fetal hemoglobin component, if collected aseptically after the birth of a healthy newborn at or near term, and whether it could be an emergency and safe substitute for adult whole blood, was the main idea behind our project.

The blood volume of a term fetus is approximately 80 - 85 ml/kg (22) The placental vessel at term contains approximately 150 ml of blood (23) The cord blood contains three types of hemoglobin, HbF, HbA, HbA2, of which HbF constitutes the major fraction (50-85 percent) (24). HbA accounts for 15 - 40 percent of hemoglobin and HbA2 is present only in trace amounts at birth (25) HbF has a greater oxygen affinity than HbA (26). The oxygen tension at which the hemoglobin of the cord blood is 50 percent saturated is 19-20 mm Hg, 6-8 mm Hg lower than that of normal adult blood. This shift to the left of the hemoglobin oxygen dissolution curve results from poor binding of the 2-3 diphosphoglycerate by HbF (27,28). The potential complications of blood transfusion therapy can be grossly divided under two headings, immunological and non-immunological reactions. (29) The immunological reactions are related to the stimulation of antibody production by the foreign alloantigens by the different components of transfusion, e.g., RBC, leucocytes, platelets and plasma proteins. Alloimmunizations may lead to immunological reactions in case of future stimulation by a similar antigen. The commonly encountered immunological
reactions are haemolytic reactions due to red cell incompatibility. Febrile or pulmonary reactions are related to antigens of leucocytes and platelets. Allergic and anaphylactoid reactions are related to antibodies and it is only very rarely that we can see graft vs. host reactions due to engraftment of the transfused lymphocytes in case of immunosuppression. The commonly encountered non-immunological reactions are because of physical or chemical properties of the transfused blood /blood products due to bacterial or viral contamination or the circulatory load.

During our experience of transfusion of 778 units of cord blood over the last 7 yrs years, we have not encountered a single episode of immunological or non-immunological reaction so far. Fetal hemoglobin can carry more oxygen than the mother's blood and there is a potential advantage of the fetal hemoglobin (Bohr's effect) by which it can carry more oxygen at low PCO2 than at high PCO2 (30). Another potent advantage of cord blood transfusion which has therapeutic implication, is the rich cytokine and growth factor filled plasma in the cord blood, which eventually has a positive effect on distressed and emaciated patients.

Continuous supply of donated blood is vital for the practice of modern medicine, but due to an ever increasing worry over blood borne diseases like HIV, hepatits or bovine spongyform encephalitis in certain areas, has fuelled the search for an alternative source for blood transfusion. Moreover, with the current global war against terrorism and other conflicts, the research to develop an ideal blood substitute has received a real boost. This has implications for not only the trauma and emergency surgeons, but the medical fraternity as a whole. Trauma surgeons, perhaps more than any other health care provider, are the first to recognize the urgency of a real blood substitute without jeopardizing the safety aspect of such a transfusion. The current generation of blood substitutes are passing through US Food and Drug Administration (FDA) Phase -III clinical testing. These include RBC substitutes to provide the respiratory functions of hemoglobin, platelet substitutes and coagulation factors. (31). The most promising among the RBC substitutes, as mentioned earlier, is the hemoglobin extracted from the lysis of the RBC from human or bovine sources, or a chemically modified hemoglobin or a genetically engineered hemoglobin molecule. Although these hemoglobin based oxygen carriers have an intrinsic advantage of universal compatibility and storability at room temperature, because of the high cost involved, these would be simply unacceptable to the developing world in particular. Moreover, there are also specific problems of hypertensive impact, gastric irritability and unexplained deaths as reported in a trauma trial on the treatment of severe hemorrhagic shock (32). The other hemoglobin substitutes with lesser importance include perflurocarbons, i.e., fluorine substituted with linear or cyclic carbon atoms with high oxygen carrying capacity, and liposome encapsulated hemoglobin (33). Transfusion of adult blood is never a zero risk event anywhere in the world. Risks associated with adult blood transfusion include transmission of HIV (1 & 2), hepatitis B, C, A, G, Parovirus 19, specially in case of pregnancy, hemolytic anemia and immunocompromised background, apart from the possibility of transfusion of syphilis, kalaazar, malaria (in the developing world), unless the blood is thoroughly screened as per WHO and country specific guidelines. There are also problems of rare blood groups which are not screened normally but have the potentiality to trigger hemolytic reactions. There are many other reasons of transfusion specific acute or delayed immunological and non immunological reactions, contamination problems with platelet, RBC, etc. Very rarely, there could be a incidence of transfusion induced lung, liver or kidney injury. Lastly, there could also be problems due to immunomodulation (34). Newly identified, but well known, potential risk factors include the possibility of the transmission of Creutzfeldt Jakob disease in its classical or variant form, even after leucodepletion (lymphocytes are possible source of transmission of
infection) as reported in an editorial article in BMJ by Mortimer P P. (35)

Attempts are being made by scientists and clinicians all over the world to make blood transfusions safer through stricter vigilance, emphasis on fewer transfusions and more conservation, preoperative autologous donation, stimulation of erythropoiesis, option for preoperative normovolemic hemodilution, attempts at intraoperative and postoperative recovery of blood, inactivation of microbes in the platelet units, use of plasma with reduced viral activity and finally, the use of red cell substitutes (36). However, in spite of all these attempted maneuvers by clinicians, the risk of transfusions remain. After our experience with 678 units of cord blood transfusion, we wish to affirm our faith in this safe transfusion protocol because we did not encounter a single case of immunological or non immunological reaction so far in any of our patients, even after the transfusion of 1 unit to 33 units (2838 ml on the basis of mean volume calculation) of cord blood to the same patient (with 10 units [mean 86x10 = 860 ml] of cord blood transfusion at a time) in different indications of blood transfusion from the pediatric to the geriatric age group (2 yrs to 86 yrs) in the common background of anemia with malignant or autoimmune or traumatic (surgical or non surgical), infective or congenital background disease (as in case of thalassemia). Our experience suggests that this placental cord blood transfusion could be an unique untapped source of fresh, infection free whole blood. If collected aseptically after the birth of healthy newborns from consenting mothers, and it has all the potentialities to be a ready replacement for blood loss.

In this connection it is worth mentioning another recent collaborative work of the University of Liverpool, U.K., and Komfo Anokye Teaching Hospital at Kumashi, Ghana, on the use of placental umbilical cord blood. They reported a substantial decrease in the mortality of children in sub-Saharan Africa suffering from severe anemia after falciparum infection, with the use of cord blood. (37).

Autologous Cord blood transfusion:

Paxson CL Jr suggested the feasibility of autologous fetal blood collection in 1979 (38). Following puncture on the umbilical vein, fetal blood was drawn into sterile heparinized plastic syringes and aliquots were subjected to coagulation and culture studies. None of the blood samples exhibited significant growth of bacterial pathogens and all patients had normal coagulation studies at 24 hours of age. These data demonstrate that fetal blood can be safely collected and given to infants subjected to shock or iatrogenic blood loss.

Almost 65% of all premature neonates with a birth weight <1,500 g receive at least one erythrocyte transfusion during their first weeks of life. The target groups of neonates who are most likely to benefit are infants with a birth weight between 1,000 and 2,000 g and neonates requiring surgical intervention directly after birth.

Another investigator group (39) claimed the use of the first newborn infant to benefit from this method of transfusion. The premature infant received two portions of autologous blood (on days 5 and 7). No untoward effects were noted. Blood, collected from the umbilical cord, is a safe source for autotransfusion, provided that bacteriological testing has been carried out. Subsequently the author concluded that the preparation of autologous RBCs from the Cord Blood of preterm infants is technically possible in principle.
In another series Brune et al.(40) reported that a comparison of cord blood and adult blood transfusion in neonates show no difference in efficacy and safety between Placental blood transfusion and allogeneic RBC transfusion. According to well-defined criteria, 40 percent of anemic neonates can be supported by autologous placental blood transfusions alone.

In another report.(41) on the neonatal surgery Taguchi T et al opined that the allogenic blood transfusions have a risk of infection owing to unknown organisms, graft-versus-host reaction, and immunosuppression; however, the use of autologous blood has been reported to be safe. Furthermore, autologous cord-blood transfusions have been reported to be effective for the treatment of anemia in premature infants. In their series the authors examined the efficacy of autologous cord blood transfusion in neonatal surgical patients and concluded that autologous cord-blood transfusion has the potential to be a useful alternative to homologous transfusion in newborns requiring surgery.

Combating anemia in paediatric age group with allogenic cord blood transfusion:

Hassal.O et al.(42) published a report on the use of allogenic cord blood transfusion to combat severe anemia in the background of malaria in pediatric age group in sub-Saharan African population. Mean volume of each blood sample obtained from the umbilical cord was 85 mL (SD 28.0). This amount of blood is sufficient to raise the haemoglobin concentrations of 28 (21%) of 131 children needing transfusions in the same hospital, by 30 g/L. There was no complications related to transfusion in the series.

(9) Bhattacharya N et al,"The safe use of Placental umbilical cord whole blood transfusion in patients suffering with anaemia and Thalassemia in underresourced regions of


(19) Hannah Hoag, "Blood substitute from worm show promise-hemoglobin from sea creature could replace red cells"4th June 2003 http://www.nature.com/nsu/030602/030602-7html


A preliminary study of placental umbilical cord whole blood transfusion in under resourced patients with malaria in the background of anaemia

Background

Malaria, caused by infection with *Plasmodium falciparum*, kills over 1 million people each year [1]. Anaemia due to malaria is a major health problem in endemic areas, particularly for young children and pregnant women. This anaemia is caused by excess removal of non-parasitized erythrocytes in addition to immune destruction of parasitized red cells and impaired compensation for this loss by bone marrow dysfunction. Though *P. falciparum* is the predominant cause of anaemia and its complications, *Plasmodium vivax* can also cause anaemia and thrombocytopenia requiring hospitalization, although to a much lesser extent.

To combat severe anaemia, several options are available: concentrated fresh red blood cell (RBC) transfusion, erythropoietin injection, blood substitutes (oxygen carriers like perflurocarbon compounds, etc), dietary supplementation of haematinics along with other essential nutrient support needed for proper erythropoiesis.

The problem, however, lies in the availability of properly screened blood, in many areas of the developing world. The cost and complications of erythropoietin therapy, which has fuelled the continued search for an ideal blood substitute, is an added difficulty. In a report of the World Health Organization, it was observed that there are about 500,000 pregnancy-related deaths globally, of which at least 25 percent maternal deaths are due to the loss of blood [2]. An estimated 13 million units of blood worldwide are not tested against human immunodeficiency viruses or hepatitis viruses, and in some developing countries 80 percent of the blood supply comes from paid donors or replacement donors (family friends or acquaintances) even when the virus-infected population is high [3].

Concerns about the safety and adequacy of the blood supply have fostered twenty years of global research into the so-called "blood substitutes" among them the oxygen carriers based on modified hemoglobin.

Foetal haemoglobin is a natural stress response to haemoglobin synthesis, which may be augmented in case of thalassemia by hydroxyurea treatment. Other conditions like pregnancy, diabetes, thyroid disease or anti-epileptic drug therapy, can also increase the foetal haemoglobin concentration. Placenta is an abundant source of foetal haemoglobin and placentas are an unused resource: in India alone, there are more than 20 million placentas produced every year of which 99.9% are discarded.

Materials and methods

Whether foetal haemoglobin-rich placental umbilical cord whole blood (which has the potential to carry more oxygen to the tissue Vol/Vol than adult blood, because of its foetal haemoglobin component) can be a safe substitute for adult blood, if collected aseptically after the birth of a healthy newborn at or near term, is the main scientific query behind the present study. Human placental umbilical cord blood was collected from consenting mothers aseptically after lower uterine Caesarean section under general or regional anaesthesia. If there was gross prematurity or dysmaturity or the projected weight of the foetus was less than 2 kg, or if the mother was suffering from any specific disease like hepatitis or HIV, etc., the cord blood collection was abandoned. The collection process started only after the
baby was safely removed from the operation field. Another sample of the cord blood collected from the placenta was immediately tested for blood group (Rh and ABO), Human Immunodeficiency Virus (HIV 1 and 2), hepatitis B and C, VDRL, malaria, bacterial and fungal infections. When the collection was complete, the blood bag tubing was closed, sealed, and transfused to a malaria victim as early as possible as per standard blood transfusion protocol, as previously reported [4-6]. Host bilirubin, urea, creatinine, glucose, were also tested along with Hb/TC/DC/ESR, before transfusion and the tests were repeated 72 hours after transfusion in some randomly selected cases to see the effect of transfusion on the rise of haemoglobin, as well as the impact on the host’s hepato-renal and metabolic profile, using standard methods.

If a patient with confirmed malaria, whose haemoglobin was less than 8 gm/dl, was not readily available, the cord blood was stored between 1°C and 4°C. This blood was transfused within three days of collection to a malaria patient with anaemia as per specifications mentioned earlier, following the World Health Organization (WHO) standard adult blood transfusion guidelines and strictly adhering to the institutional ethical committee rules and the patient consent protocol.

Results and discussion

39 patients with confirmed malaria were randomly selected for the present study after approval was given by the Institutional ethical committee for each case and the voluntary patient consent protocol was followed. The age of the patients varied from 8 to 72 years, (mean 39.4 years), of whom 24 were male and 15 were female. In this series, twenty-two patients were infected with *P. falciparum* and seventeen had *P. vivax* infection.

The 94 units of cord blood (52 ml to 143 ml in volume, mean 81 ml + 6.6 ml SD, median 82 ml, mean packed cell volume 48.9 + 4.1 SD, mean haemoglobin concentration 16.4 Gm percent + 1.6 Gm percent SD) were transfused to the 39 informed, consenting patients from 1 April 1999 to April 2005. Two units were transfused at a time to individual patients. The recipient who got the maximum amount received six units of placental blood.

The pathophysiology [7], iron metabolism [8,9] and erythropoietin production [10] in case of anaemia in chronic disease is different. The amount of transfusion depended on the severity of anaemia and the availability of compatible and screened cord blood. The pre-transfusion haemoglobin in the malaria infected patients in this study varied from 5.4 gm/dl to 7.9gm/dl for falciparum infection and 6.3gm/dl-7.8 gm/dl in vivax-infected patients. The rise of haemoglobin as estimated after 72 hours of the transfusion of two units of cord blood was 0.5gm/dl to 1.6gm/dl (Figure 1). What is interesting is the fact that there is a slow but sustained rise of haemoglobin on the seventh day after transfusion (series 3). A univariate analysis using Fishers’ exact test was performed for the results of series 2 (rise of haemoglobin after 72 hours from pre-transfusion value) and series 3 (rise of haemoglobin after 7days from pre-transfusion value). The difference between Group 2 and 3 values and its comparison with the pre-transfusion haemoglobin appeared to be statistically significant (p-less than .003). This effect could be due to the bone marrow stimulating effect of the different cytokine systems of the placental blood (Figure 2). No immunological or non-immunological reaction or adverse metabolic impact on the recipient has been encountered so far. There was no detected rise of serum creatinine (Figure 3), urea (Figure 4), glucose (Figure 5), bilirubin (Figure 6), on the recipients of two units of cord blood, when compared to the pre-transfusion level. There was also an improvement of appetite and a sense of well being in all the recipients of cord blood transfusion.

Conclusion

The placenta is a rich source of cord blood (at term there is up to 150 ml of blood in placental circulation). Cord blood is protected from infection as a result of nature’s finest biological sieve [11, 12], i.e., the placenta and contains 60-80% foetal haemoglobin (which can carry 60% more haemoglobin than adult haemoglobin) and has also a high WBC and platelet content, is hypoantigenic in nature, and has an altered metabolic profile. It may also have the potential, due to its rich
cytokine and growth factor content [13], to play a role in immune response modification in chronic anaemia.

In the developed world, umbilical cord blood (UCB) is now accepted as an alternative source for haematopoietic stem cells for transplantation, especially in children, in view of its many practical advantages. Currently there are about 100,000 units available worldwide [14]. The high oxygen affinity and anti-malarial effect of foetal haemoglobin in cord blood are additional advantages for transfusion in malaria patients with anaemia [15].

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References


Legends and Figures

Figure 1: shows the graphical impact of 2 units of cord blood transfusion on the host after 72 hours.
Series 1 shows the pre-transfusion haemoglobin in Gm/dl
Series 2 is shows the post-transfusion haemoglobin in Gm/dl (after 72 hours)

Figure 2: shows the graphical impact of 2 units of cord blood transfusion on the host after 72 hours and 7 days.
Series 1 shows the pre-transfusion haemoglobin in Gm/dl
Series 2 shows the post-transfusion haemoglobin in Gm/dl after 72 hours
Series 3 shows the post-transfusion haemoglobin in Gm/dl after 7 days

Figure 3: shows the graphical impact of 2 units of cord blood transfusion on the host’s creatinine level as seen after 72 hours.
Series 1 shows the pre-transfusion Creatinine in mg/dl
Series 2 shows the post-transfusion Creatinine in mg/dl after 72 hours

Figure 4: shows the graphical impact of 2 units of cord blood transfusion on the host’s urea level as seen after 72 hours.
Series 1 shows the pre-transfusion Urea level in mg/dl
Series 2 shows the post-transfusion Urea level in mg/dl after 72 hours

Figure 5: shows the graphical impact of 2 units of cord blood transfusion on the host’s glucose level as seen after 72 hours.
Series 1 shows the pre-transfusion glucose in mg/dl
Series 2 shows the post-transfusion glucose in mg/dl (after 72 hours)

Figure 6: shows the graphical impact of 2 units of cord blood transfusion on the host’s bilirubin level as seen after 72 hours.
Figure 1

Effect of 2 Units of Cord Blood transfusion on host's hemoglobin concentration

Randomly selected number of patients

Hemoglobin concentration in Gm/dl

Series 1
Series 2
Series 3
Figure 2

Effect of 2 Units of freshly collected cord blood transfusion on the serum Creatinine level of the host

<table>
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<th>Serum Creatinine in mg/dl</th>
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Figure 3

Effect of 2 Units of freshly collected cord blood transfusion on the host’s blood Urea level

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Figure 4

Effect of 2 Units of freshly collected cord blood transfusion on host’s glucose level in mg/dl after 72 hours

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<th>Host blood glucose level in mg/dl</th>
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Experience of cord blood transfusion in diabetes, anemia and microalbuminuria

Background:
Anemia is a common accompaniment of diabetes, particularly in patients with albuminuria or reduced renal function. The estimated prevalence of anemia depends on an essentially arbitrary criteria used to define the presence or absence of anemia. Anemia in the background of chronic disease is mostly immune driven, where cytokines and cells of the reticuloendothelial system participate in altering the iron homeostasis. They also prevent adequate proliferation of erythroid progenitor cells, dysregulation of erythropoietin (Epo) production and sensitivity, leaving aside the problem of the altered life span of red cells (1). When a patient with a chronic metabolic disease like diabetes reports with anemia, many factors have been suggested as the reason for the onset of anemia. A lower Hb count is significantly associated with a more rapid decline in the glomerular filtration rate (GFR) (2). Furthermore, treating anemia early in renal failure has been demonstrated to slow the rate of decline in renal function (3). One of the most potent causes of sub-optimal response to Epo is chronic and overt inflammation, associated with an increased production of cytokines, such as tumor necrosis factor-α, interleukin-1, or interferon-γ (4), which might suppress erythrocyte stem cell proliferation (5). Anemia also has a negative impact on patient survival, and is considered to be an important cardiovascular risk factor associated with renal disease. It appears more likely that proteinuria is a marker of tubulointerstitial injury in diabetes (6), perhaps more so than in non-diabetic conditions associated with proteinuria, which is considered to be primarily glomerular in origin. It has been suggested that the widespread use of ACE inhibitors may contribute to anemia in patients with diabetes (7). Excretion of growth factors in the urine has been implicated in the pathogenesis of tubulointerstitial disease that characterizes proteinuric renal disease. Understanding the pathogenesis of anemia associated with diabetes and nephropathy may therefore lead to opportunities for developing interventions to optimize outcomes in these patients.

In order to combat severe anemia (Hemoglobin 8gm/dl or less), there are several options: concentrated fresh RBC transfusion or erythropoietin injection, or blood substitutes (oxygen carriers like perfluorocarbon compounds, etc), apart from dietary supplementation of hematinics along with other essential nutrient support needed for proper erythropoiesis.
The real problem lies in the availability of proper screened blood in nano or molecular level. This is a difficult task even in most areas of the developing world. Apart from this, the cost and complications of erythropoietin therapy have fuelled the continued search for an ideal blood substitute.

The placenta is a readily available source of fresh whole blood. It can be collected aseptically after lower uterine Caesarean section (LUCS) and tested according to the standard adult blood screening procedure. This placental blood is rich in fetal hemoglobin and has the potential to carry more oxygen than adult hemoglobin to the tissue Vol/Vol after the birth of a healthy newborn at or near term, because of its fetal hemoglobin component. The formidable placental barrier is one of the finest biological barricades, which protects the baby from infection till term.

This placenta, or the after birth, is discarded routinely everywhere, and is actually a cause of environmental pollution in many parts of the developing world (in India alone, there are more than 20 million placentas produced as afterbirth every year), because it attracts natural scavengers and spreads infection unless aseptically treated or incinerated. My team of doctors has been successfully transfusing placental cord whole blood, which is rich in fetal hemoglobin content, as an alternative emergency source of blood transfusion in the background of anemia and emaciation of any etiology (8,9).

Materials and methods:

Whether fetal hemoglobin rich placental umbilical cord whole blood, with its various unique features, can be an emergency and safe substitute for adult whole blood in case of diabetes with anemia, with hemoglobin concentration of less than 8 gm percent, was the main idea behind the present study. This research was funded by a grant from the Department of Science and Technology, Government of West Bengal, for a study on cord blood (1999-2002). In this study, 39 informed, consenting patients (22 male= 17 female, age 48-74 yrs, mean 59.6 yrs) were included. The patients were randomized into two groups: Group A (Control cases N=15, male =8 and female =7) and Group B (study group N=24, male=14 and female =10). Group A (N=15) were treated according to the standard regime of act rapid insulin, ace- inhibitor, combating dyslipidaemia, hyperuricosuria, and fresh adult rbc transfusion 2-4 units each, depending on availability. Group B (N=24) patients were treated with an identical regime but freshly collected cord blood was transfused instead of adult blood 2-4 units each, depending on availability after cross-matching and fulfilling other essential criteria.

The Type 2 diabetes patients who were enrolled in the present study were clinically examined and standard indices were recorded from the tested blood, including creatinine, urea, albumin, fasting blood glucose, fasting lipid profile, HbA1c, C-reactive protein and ferritin. Urinary creatinine, urea, albumin, and protein were obtained from a 24-h collection were also recorded. Medical records of these patients showed no evidence of advanced diabetic nephropathy (creatinine clearance > or = 30 mg/kg/1.7 m²).

For inclusion in this study, the patient’s plasma hemoglobin had to be 8gm percent or less (the pre transfusion hemoglobin in this series varied from 5.2 gm/dl to 7.8gm/dl) in the background of Type 2 diabetes (fasting sugar 200mg or more) along with features of microalbuminuria (albumin excretion 30-299 mg/g creatinine).

78 units of human placental umbilical cord blood were collected from consenting mothers aseptically after lower uterine Caesarean section under general or regional anesthesia. The collected blood volume varied from 56ml -138 ml, mean 82 ml±5.6 ml SD, median 84 ml, mean packed cell volume 49.7 + 4.2 SD, mean hemoglobin concentration 16.6 Gm percent + 1.5 Gm percent SD. If there was gross prematurity or dysmaturity or the projected weight of the fetus was less than 2 kg, or if the mother was suffering from any specific disease like hepatitis or HIV, etc., the cord blood collection was abandoned. When the collection was complete, the blood bag tubing was closed, sealed, and stored at 1- 4 degree centigrade, after putting necessary identification markings. Another sample of the cord blood collected from the placenta was immediately tested for blood group (Rh and ABO), HIV (1 and 2), hepatitis B and C, VDRL, malaria as per standard blood transfusion protocol, on which we have reported earlier (10,11).
Result and discussion:
The collected cord whole blood was transfused as early as possible, latest by 72 hours of collection, to a diabetic patient with anaemia in Group B, after grouping, cross matching and following the standard adult blood transfusion WHO guideline for transfusion. There was strict adherence to the institutional ethical committee guidelines and the patient consent protocol in all cases.

The data showed that in Group A there was an increase in the Hb count after 2 units of adult blood transfusion, from 1.5 to 1.8 gm/dl. The rise of hemoglobin, as estimated after 72 hours in Group B, after 2 units of cord blood transfusion, was .5gm/dl to 1.6gm/dl. There was also a subjective improvement of appetite and a sense of well being in all the recipients of cord blood.

Microalbuminuria was assessed in both groups after one month of treatment with transfusion and other identical support. The mean value of albumin excretion in 24 hours urine was 152 + 18 mg S.D of albumin per gm of creatinine (the pre-transfusion mean was 169 + 16 mg) in Group A and 103+16mg S.D per gm of creatinine in Group B (the pre-transfusion mean value was 193+21 mg). A univariate analysis using Fishers’ exact test was performed for the results of Group A and Group B. The differences in Group A and B values of microalbuminuria appeared to be statistically significant (p<less than .003).

The most important cause of end state renal disease is diabetes. It causes a decline in the production or receptor sensitivity or dysregulation of the excretion of erythropoietin This Epo concentration may provide an indirect evidence of the functioning renal status. In some cases of uncontrolled diabetes, there is an unexplained anemia which could be due to a low serum level, or blunted response to erythropoietin as a result of interstitial damage, or an abnormal glycosilation of the cytokine system, dysautonomia, or presence of infection only to name a few important causes (12,13,14,15,16,17,18,19,20,21,22,23). Anemia is very common in diabetes and potentially contributes to the pathogenesis of different diabetic complications. Independent predictors of anemia in diabetes are transferrin satuation, glomerular filtration rate, sex, albumin excretion rate, glycosilation status, etc (24). In case of anemia in chronic disease there is an acute or chronic immune activation of a specific cytokine system, which helps in shifting of the iron from its normal route. The condition has also been termed as “anemia of inflammation” (25). This condition is immune driven. It could also be due to pro-inflammatory cytokines and free radicals that damage erythroid progenitor cells. Bleeding episodes, vitamin deficiencies (e.g., of cobalamin and folic acid), hypersplenism, hemolysis, helminthiasis, malnutrition, may all contribute to the anemic process as well. Hepcidin, an iron-regulated acute-phase protein that is composed of 25 amino acids, has helped to shed light on the relationship of the immune response to iron homeostasis and anemia of chronic disease (26, 27).

Blood transfusion is an option to tackle severe life threatening anemia. Another option to tackle anemia is to inject Epo provided there is no dearth of iron or B12 store. However, there is little data currently available on the possible effects of Epo on the course of the underlying disease, particularly since it may exert additional biologic effects, including interference with the signal-transduction cascade of cytokines (28).

In the underprivileged world many patients treated in government hospitals cannot afford to buy Epo to prevent renal deterioration and correct the anemic condition, and in many situations they cannot even arrange for fresh whole blood or concentrated RBC . These are the cases which can benefit from cord blood transfusion. Considering the insufficient health infrastructure in developing countries, their poorly trained manpower facilities, inadequate screening and cost of screening of blood at the nano level for transfusion, we tried to solve the problem through our own resources. In India alone, more than 20 million registered births take place every year.

The placenta is a rich source of cord blood (at term there is up to 150 ml of blood in placental circulation). It also has a unique microenvironment and has a sensitization impact on cord blood cells. The placenta is a complex organ that regulates maternal-fetal interactions. Many cytokines that can influence lymphohematopoietic development, e.g., G-CSF (Granulocyte colony stimulation factor), c-kit ligand (stem cell factor [SCF]), GM-CSF (Granulocyte macrophage colony stimulated factor), IL-15 (Interleukin 15), and others, are produced by the placenta. G-CSF is produced both by the maternal decidua and the fetal chorionic villi and enters the fetal circulation by a process that does not require a functional G-CSF receptor. G-CSF from the mother probably does not enter the fetal circulation. An experiment has demonstrated that the administration of recombinant human G-CSF (rhG-CSF) to pregnant macaques did not result in detectable rhG-CSF in the fetuses (29). The function of placental G-CSF production is unknown; however, it may serve as an immunoregulator that protects the mother...
and fetus from each other’s allogeneic immune systems. G-CSF inhibits the ability of placental mononuclear cells to mediate cytotoxicity against allogeneic targets including choriocarcinoma cells.

Freshly collected cord blood, rich in hemoglobin and growth factors, may have a positive impact on the anemia of chronic disease. All the patients irrespective of their background in our present series tolerated the procedure well and there was a sense of well being in most of the cases, as mentioned earlier.

The exact aetiopathogenesis behind the improvement of the microalbuminuria in the cord blood transfusion (Group B) is not clear, apart from the fact that freshly collected cord blood is rich in its content of many cytokine or growth factors whose individual impact is currently under study. Fetal hemoglobin rich cord blood with its altered viscosity, may have a positive impact on renal perfusion. Cord blood has a high WBC and platelet content, is hypoantigenic in nature, and has an altered metabolic profile. It may also have the potential to play a role in immune response modification in chronic anemia (which we are studying at present), due to its rich cytokine and growth factor content.

Our group of researchers is working on the problem of fetal cell or tissue transplant in the adult system. We are also working on the use of umbilical cord whole blood transfusion as an alternative to adult whole blood transfusion from the pediatric to the geriatric age group in different indications, since 1st April 1999, and have published our reports from time to time (30,31,32,33,34,).

Conclusion

Cord blood may also be a safer alternative in the contemporary context. Although transfusion of adult blood may decrease short-term mortality, the risk of human immunodeficiency virus (HIV) transmission is considerable in Africa and many parts of Asia. Transfusion-associated AIDS accounts for 10 percent of all cases of AIDS in Africa. The risk of HIV-1 contamination in transfusions continues to exist, even in countries where blood products are screened, because of limitations in test sensitivity, human error, and the window period. Furthermore, 30 African countries do not screen all of their blood products because of resource limitations. Decision analysis should be used to compare survival outcomes of severely anemic patients who receive transfusions against those who do not. Results indicate from African studies that when 5 percent of the blood supply is HIV-1 contaminated, everyone with 6.6 percent or more risk of dying from anemia should be transfused (35).

In this context, it should be noted that the placenta is an unique and formidable biological barrier which protects the conceptus till term. There are many substances like P-glycoprotein, which forms a functional barrier between maternal and fetal blood circulation in the placenta, thus protecting the fetus from exposure (36). Even HIV cannot cross this barrier easily. However, at or near term there is an increase in the fetomaternal bi-directional traffic as some cells may have access to the maternal circulation depending on the viral load pathogenicity of the virus, the maternal immune condition and many more hitherto identified and non identified factors. One investigating group has suggested that the trophoblastic barrier remains uninfected in full-term placentae of HIV-seropositive mothers undergoing antiretroviral therapy. They suggested that in utero, HIV transmission, if at all, occurs at the end of gestation through alternative routes, such as chorioamnionitis with leakage of the virus into the amniotic cavity or trophoblast damage (37).

In the developed world, umbilical cord blood is now accepted as an alternative source for hematopoietic stem cells for transplantation especially in children, due to its many practical advantages. It is an alternative source of stem cells and is easy available. Collection of this blood is without any risk for mothers. There is less possibility of infection, particularly cytomegalovirus; reduced risk of GVHD, with easy HLA matching criteria for donor-recipient selection. UCB banks have been established for related and unrelated UCBT with about 100,000 units currently available (38). The use of cord blood can save life in case of severe anemia in underresourced children of Africa (39) or any other part of the world.

However, these centers which use cord blood for transplantation purposes, use only .01 percent of the cord blood, i.e., the stem cells only, and discard 99.99 percent. In this preliminary communication
of our work with cord blood in malaria patients whom we have followed up for 6 years, we have seen that properly screened freshly collected cord blood transfusion is safe in diabetic patients who are also suffering from anemia. It improved the hemoglobin content of all the patients in our study. This may perhaps be due to the transfusion of the cord blood itself, which as noted, is rich in fetal hemoglobin, or because of the cytokine or growth factor impact on the recipients’ bone marrow and kidney, which may have antagonized the chronic or inflammatory anemia and the erythropoietin deficiency in supply, or receptor sensitivity caused by tubulointerstitial injuries in diabetic patients. Proteinuria is not only a major correlate of declining renal function but may also directly lead to disease progression by contributing to tubulointerstitial injury due to the release of inflammatory and vasoactive substances into the interstitium (40). The enhanced ultrafiltration of growth factors that occurs in proteinuric states has also been implicated as pathogenetically linked to the development of tubulointerstitial disease (41,42). The improvement of microalbuminuria in Group B patients may be a result of the positive effect of the pregnancy-specific growth factors and cytokine systems on the renal derangement caused by diabetes. The transfusion of hypoantigenic cord blood also did not trigger any immunological or non-immunological reaction. Hence, it appears that freshly collected cord blood transfusion is not only a transfusion of fetal hemoglobin rich high oxygen affinity blood, but also an infusion of serum which is rich in pregnancy specific growth factors and cytokines. In diabetic patients with anemia, its promise could be immense, not only in under-resourced countries where patients are cash-strapped, but also in developed countries, where patients may benefit from the extra potentials of cord blood.

REFERENCES


Introduction

Tuberculosis (TB) represents an important worldwide health hazard. It has been reported by the World Health Organization (WHO) that one person in the world becomes infected every second, and that one third of the entire population of the world is now infected. The World Health Organization also estimates that in the next decade, 300 million more people will be infected, 90 million people will develop the disease, and 30 million people will die from it (1). Among those aged over 5 years, tuberculosis kills more people than AIDS, malaria, diarrhoea, leprosy, and all other tropical diseases combined. The tragedy of this situation is that treating tuberculosis today is one of the most effective and cost-effective of all health interventions. As per the WHO estimate the annual number of deaths could be from 3 million to 4 million by the year 2004 (2). Patients with persistent pulmonary infections from mycobacterial diseases present a difficult clinical challenge. These individuals typically have poor pulmonary function, malnutrition, and other co-morbidities, and few guidelines exist regarding optimal therapy. The prevalence of tuberculosis infection and clinical disease varies widely in different age groups. However, among children in household contact with adult patients, it is higher than in the

Placental umbilical cord whole blood transfusion to combat anemia in the background of tuberculosis and emaciation and its potential role as an immuno-adjuvant therapy among the under-resourced people of the world
general population, and the risk is significantly increased by contact with sputum positive adults. The nutritional history of patients with TB has revealed that they have higher levels of anorexia, vomiting, nausea and diarrhoea. Consequently, both male and female TB patients suffer from considerable malnourishment. It has been recommended that these patients should receive nutritional support during their treatment, with studies of the exact nutritional deficiencies at the micronutrient level and their effect on the immune system being required. Directly Observed Treatment-Short Course (DOTS) has been a successful strategy in the global control of tuberculosis in adults. However, there are uncertainties of TB in extremes of ages, i.e., in the pediatric and geriatric age groups, as well as in adults who receive steroids and other immunosuppressives, or in the case of uncontrolled diabetes, or in nutritionally deprived patients in the under resourced regions of the world. In such cases, patients can present to a physician with vague clinical symptoms, unreliable tuberculin tests or TB score charts, non-specific hematological, biochemical or radiological evidences, difficulty in sputum expectoration and non-availability or ill-affordability of specialised tests.

Anemia in tuberculosis is a serious co-morbidity, which is caused by various factors, for instance, hemoptysis in cases of pulmonary Kochs in advanced stages, or it could occur as a result of lack of proper nutrition and micronutrients in the diet, or coexistent helminthiasis, and/or other coexistent diseases like HIV and/or other preexisting or compounding gastrointestinal problems, which alter the available iron store or reserve or cause bone marrow dysfunction. In the Indian subcontinent, malnutrition and anemia, weakness with evening rise of temperature and an emaciated look are a quite typical presentation in case of persons with tuberculosis in the rural and semi-urban areas, who report to state government hospitals for a free supply of medicines and other essential drug support.

My team of doctors has been successfully transfusing placental cord whole blood, which is rich in fetal hemoglobin content as well as cytokine and growth factors, as an alternative emergency source of blood transfusion in the background of anemia and emaciation of any etiology. The placenta, or the after birth, is discarded routinely everywhere in the world (in India alone, there are more than 20 million placentas produced as afterbirth every year), and is actually a cause of environmental pollution in many parts of the developing world because it attracts natural scavengers and spreads infection, unless aseptically treated or incinerated. The centers of excellence in the western developed world have been working on the use of a tiny microscopical fraction of cord blood, i.e., CD 34 stem cells only (.01 percent of the nucleated cells of the placental blood). Whether fetal hemoglobin rich placental umbilical cord whole blood (which has the potential to carry more oxygen to the tissue Vol/Vol than adult blood because of its fetal hemoglobin component, if collected aseptically after the birth of a healthy newborn at or near term), could be an emergency and safe substitute for adult whole blood in case of tuberculosis victims with hemoglobin concentration of less than 8 gm percent, was the main idea behind the present study. We received a grant (1999-2002) from the Department of Science and Technology Government of West Bengal.

Material and Methods

This is a government hospital based study conducted in Calcutta (India), where most poor patients are admitted in a free bed to receive free treatment. This study included marginalized persons, i.e., homeless persons, alcoholics, migrants, drug abusers, landless labourers and the poor from any strata of society. We enrolled patients from our hospital who were suffering from anemia, tuberculosis, emaciation and who could not buy erythropoietin or arrange for fresh whole blood or concentrated RBC, for cord blood transfusion. All enrolled cases gave proper informed consent and were passed through the institution based ethical committee.

Materials and methods:
106 units of human placental umbilical cord blood were collected from consenting mothers aseptically after lower uterine Caesarean section under general or regional anesthesia. The volume varied from 48ml -148 ml, mean 81 ml±6.6 ml SD, median 82 ml, mean packed cell volume 49.4 + 3.1 SD, mean hemoglobin concentration 16.3 Gm percent + 1.7 Gm percent SD. After collection the blood was immediately tested and transfused or preserved in the refrigerator and transfused within 72 hours of collection if no recipient was available at that time. This study is ongoing from 1st April 1999. In case of gross prematurity or dysmaturity, or if the projected weight of the fetus was less than 2 kg, or if there was any specific disease affecting the mother like hepatitis or HIV, etc., the cord blood collection was abandoned. Cord blood was collected from only informed, healthy mothers with their consent after the birth of their healthy babies. The collection process started only after the baby was safely removed from the operation field and the anesthetist verified the stable physical condition of the mother. It was only then that the obstetrician took the decision to proceed with the umbilical cord blood collection. Immediately, the cord was disinfected by spirit/Betadine solution at the site of the proposed puncture of the umbilical vein and a 16 g needle was attached to a standard pediatric collection bag (containing 14ml anticoagulant citrate phosphate dextrose adenine solution), which was used for the purpose of collection. When the collection was complete, the blood bag tubing was closed, sealed, and stored at 1-4 degree centigrade, after putting necessary identification markings. Another sample of the cord blood collected from the placenta was immediately tested for blood group (Rh and ABO), HIV (1 and 2), hepatitis B and C, VDRL, malaria, fungus and bacterial study, as per standard blood transfusion protocol, on which we have reported earlier (7,8). The collected cord whole blood was transfused immediately or at the most, within three days of collection to a patient with anaemia vide (Fig 1 ), after grouping, cross matching and following the standard adult blood transfusion WHO guideline for transfusion, and simultaneously, strictly adhering to the institutional ethical committee’s instructions and the patient consent protocol. Pre-transfusion, and three days after the transfusion, blood was drawn from the consenting patients for peripheral blood hematopoietic stem cell estimation (CD34) by flow analysis cytometry as per standard protocol at Ranbaxy Laboratory. This procedure was repeated after three months for comparison.

Results and discussion:

As mentioned earlier, tuberculosis is the one of the most important chronic diseases in the world and a leading infectious cause of millions of deaths annually (9). Varying degrees of anemia are very much prevalent in tuberculosis patients, which could be due to background malnutrition, coexistent diseases like helminthiasis or drug impact on the immune system (including the bone marrow), and poor red cell survival.

The rationale for the treatment of anemia in chronic diseases is based on two principles. First, anemia can be generally deleterious in itself, requiring a compensatory increase in cardiac output in order to maintain systemic oxygen delivery; second, anemia is associated with a poorer prognosis in a variety of conditions. Thus, moderate anemia warrants correction. Blood transfusions are widely used as a rapid and effective therapeutic intervention in anemia. Transfusions are particularly helpful in the context of either severe anemia (in which the hemoglobin is less than 8.0 g per deciliter) or life-threatening anemia (in which the hemoglobin is less than 6.5 g per deciliter), particularly when the condition is aggravated by complications that involve bleeding.

We have transfused freshly collected 106 units of placental umbilical cord whole blood within 72 hours of collection from consenting patients undergoing lower uterine caesarean section maintaining standard WHO specified blood transfusion norms for our country. Each case has been followed up till date.

Twenty-one patients with anemia (8Gm per deciliter or less) in the background of tuberculosis were included in this study. Sixteen cases were suffering from pulmonary Kochs', and presented with cavitation in 4 cases. The rest – 5 cases – had extra-pulmonary Kochs' involvement, i.e., intestinal Kochs' involvement was detected in 4 cases and skin involvement in 1 case.
The criteria for clinical diagnosis of tuberculosis were typical clinical features like loss of weight, evening rise of temperature, weakness and other constitutional symptoms depending on the primary involvement of the organ. For assessment of the Kochs’ status, primary or reactivation and the background, X-rays were taken, and usual tests like Mantoux test, Hemoglobin, total count of WBC, differential count of WBC and erythrocyte sedimentation rate, were done. However, in extra-pulmonary and non hemoptosis, silent presentation of suspected Kochs’ infection, Elisa TB IgA, IgM, IgG and screening for HIV 1 and 2 were done routinely in young age group. In case of clinical confusion, adenine deaminase, gamma interferon, fine needle aspiration cytology and biopsy were used as supportive investigation. In two cases, we had to take the support of DNA (PCR) from ascitic fluid and also from serum, for confirmation. In the pulmonary presentation group, in addition of Kochs’ reactivation, two cases had HIV in the background and 4 cases had cancer. The group included 13 female patients and 8 male patients with their ages varying from 18 years to 74 years. So far, a total of 106 units of freshly collected blood was transfused to these cases without encountering a single case of immunological or non immunological reaction. The patients received two units to 21 units of blood with 8 units at a row in one case, to combat anemia due to haemoptysis induced blood loss. All the patients who received this blood had positive clinical responses like less weakness, a sense of well being and weight gain, which was quite obvious in patients who received more than three units of blood (16 cases).

What is interesting, and we have not been able to trace any similar phenomenon in published medical literature, is the rise in the peripheral blood CD34 level assessment by flow analysis cytometry done 72 hours after transfusion. This test was repeated after 3 months in consenting volunteers (vide Figure 2). The normal CD34 level in the peripheral blood is .09 percent. In the present series of patients suffering from tuberculosis with anemic background, among those who received cord blood, the level of CD34 varied from 2.99 to 33 percent. This returned to individual base levels in 66.66 percent of the cases, at the three month CD34 re-estimation. The point at issue is why did this rise in the CD34 level take place, and why did it vary from individual to individual without provoking any clinical graft vs host reaction. No patient in the present series (HLA and sex randomized) received any specific immunosuppressive drug therapy apart from the anti-tubercular drug. One possible answer could be the hypo antigenic cord blood and immune mosaic condition in tuberculosis; another could be that the freshly collected cord blood contains growth stimulating cytokine, and this may have an impact on the hosts' bone marrow or any other specific system.
Figure 1:
A patient is receiving placental umbilical cord blood transfusion in presence of the investigator and his research associates. Two female patients near the head end are waiting for their turn of blood transfusion. This particular patient was getting his treatment for Kochs’ infection in the background of HIV positive status.
Figure 2: The flow analysis cytometry report (of one case) of the peripheral blood showed 8.13 percent CD34 level collected from the peripheral blood, 72 hours after the ABO/Rh group cross-matched cord blood transfusion.

In the developing world as a whole and in India in particular, it is a common everyday knowledge to a physician treating tuberculosis, that nutritional status deterioration may help in activating the latent or subclinical form of tuberculosis. Protein malnutrition has been identified as an important risk factor for the predisposition to intracellular infections leading to destabilization of the clinical condition. There is a strong association between protein malnutrition and impairment of a range of immune functions, principally those mediated by T lymphocytes, which are known to be essential for the resistance to tuberculosis.

Another characteristic is the anemia resulting from chronic disease, as seen in tuberculosis. Here, there are disturbances of iron homeostasis, with increased uptake and retention of iron within cells of the reticuloendothelial system. This leads to a diversion of iron from the circulation into storage sites of the reticuloendothelial system, subsequently limiting the availability of iron for erythroid progenitor cells, and causing iron-restricted erythropoiesis. In mice that are injected with the pro-inflammatory cytokines interleukin-1 and tumor necrosis factor α (TNF-α), both hypoferremia and anemia develop (10). In chronic inflammation, the acquisition of iron by macrophages most prominently takes place through erythrophagocytosis (11) and the transmembrane import of ferrous iron by the protein divalent metal transporter 1 (DMT1). Interferon-γ, lipopolysaccharide, and TNF-α up-regulate the expression of DMT1, with an increased uptake of iron into activated macrophages (13). The identification of hepcidin, an iron-regulated acute-phase protein that is composed of 25 amino acids, helped to shed light on the relationship of the immune response to iron homeostasis and anemia of chronic disease. Hepcidin expression is induced by lipopolysaccharide and interleukin-6 and is inhibited by TNF-α (14).
In India, tuberculosis remains the leading infectious cause of death, killing close to 500,000 people a year. India has far more cases of tuberculosis than any other country in the world — about 2 million new cases each year (15), and accounts for nearly one third of prevalent cases globally. The Indian tuberculosis-control program is now one of the largest public health programs in the world. The program has been remarkably successful, although it still faces many challenges. Direct health benefits to date include the treatment of 1.4 million patients with tuberculosis and prevention of more than 200,000 deaths (16).

Primary tuberculosis, a self-limited, mild pneumonic illness that often goes undiagnosed, may develop in a subgroup of infected persons in nutritionally deprived or immunocompromised individuals or in very young pediatric patients. During this illness, bacillemia and seeding of other organs may occur, setting the stage for subsequent reactivation in extra pulmonary sites. In USA, one investigator has suggested that in about 5 percent of persons, the infection progresses from a latent form to active disease within two years after infection, and an additional 5 percent have active disease at some later point in their lives. Although the majority of cases of active tuberculosis are thought to arise from a reactivation of latent infection, exogenous re-infection with a second strain of M. tuberculosis can occur, particularly in profoundly immunocompromised persons and in those heavily exposed to new bacilli. Nucleic acid technology provides rapid, specific, and sensitive diagnostic tests and rapid detection of drug resistance (17, 18, 19). Important clinical signs of pulmonary tuberculosis include cough, pleuritic pain, and hemoptysis. Though the lung is involved in 80 percent or more in USA, extra pulmonary sites of disease are also seen as in lymph nodes, pleura, bones or joints, the genitourinary system, the central nervous system, the abdomen and pericardium, and in rare cases, virtually any other organ. Diagnostic standards and classification of tuberculosis in adults and children have been widely discussed in medical literature (20). However, the clinical signs of pulmonary tuberculosis are more varied and less specific in persons with HIV infection (21).

Recent advances in the treatment of tuberculosis like combination therapy for tuberculosis revolutionized the outcome of the disease. But in the under-resourced world, the real problems are hunger and malnutrition, which have a very important role in reactivation of the disease. Hence, the presentation becomes uniquely compounded and the responsibility of treatment and the outcome become less positive.

For example, anemia of chronic diseases like tuberculosis is the result of acute or chronic immune activation due to inflammation. This anemia is the second most prevalent variety after anemia caused by iron deficiency (22). In severe anemia when the hemoglobin level is 8gm per deciliter or less, we often decide to give packed cell transfusion slowly. The 21 patients of our hospital who volunteered for the cord blood transfusion and received a total of 106 units of cord blood, did not encounter a single case of immunological and non immunological reaction. We consider this to be very positive news and wish to share it with the medical fraternity through this article.

What is intriguing is the rise of peripheral blood CD34 level after cord blood transfusion as seen in the flow analysis cytometry report. The reason for the transient rise of hematopoietic stem cells as seen in the peripheral blood in HLA randomized recipients without any immunosuppressive support and without provoking clinical graft vs host reaction, still remains a mystery.

However, we can venture some probable explanations. The placenta has a unique microenvironment and its sensitization impact on cord blood cells may have a role in transient transplantation impact on the host system. One very important factor, apart from intrinsic differences, is the fact that HSC (hematopoietic stem cell) in UCBC (umbilical cord blood cell) have had a different set of microenvironmental exposures compared to those of adult marrow or PBSC (peripheral blood stem cell). An example of differences between sources are some of the observed changes in HSC cell cycle status, gene expression and the adhesive and invasive properties induced by mobilization procedures used to generate PBSC, e.g., G-CSF (granulocyte colony stimulation factor), The placenta is a complex organ that regulates maternal-fetal interactions. Many cytokines that can influence lymphohematopoietic development, for e.g., G-CSF, c-kit ligand (stem cell factor [SCF]), GM-CSF (granulocyte

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macrophage colony stimulated factor), IL-15 (Interleukin 15), and others, are produced by the placenta. Production of G-CSF by the placenta may be especially relevant to UCBT. G-CSF is produced both by the maternal decidua and the fetal chorionic villi and enters the fetal circulation by a process that does not require a functional G-CSF receptor. G-CSF from the mother probably does not enter the fetal circulation, as administration of recombinant human G-CSF (rhG-CSF) to pregnant macaques did not result in detectable rhG-CSF in the fetuses (23). The function of placental G-CSF production is unknown; however, it may serve as an immunoregulator that protects the mother and fetus from each other's allogeneic immune systems. G-CSF inhibits the ability of placental mononuclear cells to mediate cytotoxicity against allogeneic targets including choriocarcinoma cells.

In fine, though precisely not clear as yet, the functional hypoantigenecity of the freshly collected and immediately transfused cord blood antigen with its complex cytokine interaction, may have a role in immune selective masking, i.e., immune mosaicism, in those anemic patients with tuberculosis who have immunosupression either due to drugs, chronic nature of the disease, malnutrition with helminthiasis, or other associated factors like the impact of growth factors or selective cytokine impact of the cord blood on the bone marrow of the recipient. All the patients irrespective of their background tolerated the procedure well and there was a sense of well being in most of the cases along with weight gain in 85.5 percent cases. There was no clinical graft vs host reaction or any other immunological or non immunological reaction among the transfusion recipients in this present study and in the follow up so far.

Our group of researchers is working on the problem of fetal cell or tissue transplant in the adult system (24, 25,26,27,28). We are also working on the use of umbilical cord whole blood transfusion as an alternative to adult whole blood transfusion from the pediatric to the geriatric age group in different indications since 1st April 1999. We are of the opinion that cord blood collected aseptically from the placenta of the consenting mother after the birth of a healthy baby, has all the potential of an effective therapeutic adjuvant for tuberculosis patients with anemia in the underprivileged world. Cord blood is protected from infection as a result of nature’s finest biological sieve, i.e., the placenta, and contains 60-80 percent fetal hemoglobin (which can carry 60 percent more hemoglobin than adult hemoglobin), and moreover, has a high WBC and platelet content, is hypoantigenic in nature, and has an altered metabolic profile. It may also have the potential (?), (which we are studying at present), to convert TH2 responses to TH1 response due to its rich cytokine and growth factor content, which may have a role in immune response modification.

Conclusion

Tuberculosis causes approximately 1.5 billion latent infections, 8 million new clinical cases and millions of deaths annually, which makes it the most prevalent infectious disease in the world (29). The incidence of tuberculosis is unusually high among malnourished people, including the elderly, the homeless, alcoholics, drug abusers, and human immunodeficiency virus-infected individuals. The detrimental effects of nutritional deficiencies on tuberculosis could result from alterations in the T lymphocytes and macrophages functional regulation, which are the major cell types mediating antitubercular immune[30]. Cytokines play a central role in mediating antitubercular immunity[31]. Other advances in our understanding of the pathophysiology of anemia of chronic diseases like tuberculosis suggested that disturbances of iron homeostasis, impaired proliferation of erythroid progenitor cells, and a blunted erythropoietin response to anemia — have made possible the emergence of new therapeutic strategies. These include treatment of the underlying disease and the use of erythropoietic agents, iron, or blood transfusions. Despite inadequate funding resources, much effort is being devoted to both approaches, involving a multidisciplinary approach from diverse disciplines such as molecular biology, social anthropology, and health economics.
Umbilical cord blood (UCB) is now accepted as an alternative source for hematopoietic stem cells for transplantation, especially in children. Unrelated UCB offers many practical advantages as an alternative source of stem cells, including: (1) easy availability without risk for mothers or donors; (2) less possibility of infection, particularly cytomegalovirus; (3) documented reduced risk of GVHD, with easy HLA matching criteria for donor-recipient selection, and (4) UCB banks have been established for related and unrelated UCBT with about 100,000 units currently available (32). But, so far, all centers in the world discard the precious umbilical cord blood after separating 0.01 percent stem cells from it. We are the only researchers in the world who are working on the use of fetal hemoglobin and cytokine rich umbilical cord whole blood transfusion as an alternative source of blood, and transfusing this umbilical cord whole blood to our volunteers suffering from tuberculosis and anemia since 1999 with the funding support of the Dept of Science and Technology, Govt of West Bengal.

The clinical improvement as a result of cord blood transfusion and the transient rise in the peripheral blood CD34 level, stimulates us to think of the probable adjuvant immunopotentiating role or immunotherapeutic impact on the hosts’ (?) suppressed immune system which had been caused by malnutrition, chronic disease or even drug impact. Hepatotoxicity occurs with isoniazid, rifampicin, pyrazinamide and ethionamide. Risk factors include old age, malnutrition and high alcohol consumption. If cord blood has an adjuvant immunotherapeutic impact, it may play a positive role in negating immunosuppression in TB patients.

In this preliminary communication after following up for 6 years, we wish to note that the use of cord blood transfusion in tuberculosis patients with anemia is not only safe, but the unique phenomenon of transient spontaneous transplant impact of the CD34 cells, as seen in the peripheral blood after cord blood transfusion in HLA randomized recipients, without the support of the immunosuppressives, without provoking clinical graft vs host reaction is an interesting positive finding. This has never been cited earlier in published medical literature and we wish to communicate this finding to our medical fraternity so that further research may be undertaken.

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Placental umbilical cord whole blood transfusion to combat anemia in the background of advanced rheumatoid arthritis and emaciation and its potential role as an immuno-adjuvant therapy

Introduction:

Anemia is a common comorbidity in individuals with rheumatoid arthritis (RA). In fact, anemia of the type characterized by low serum iron concentrations in conjunction with adequate iron stores is frequently associated with RA and has served as a model for anemia of chronic disease. Investigators have suggested that patients with RA who have anemia, are likely to have more severe joint disease, and if the anemia is successfully treated, the joint disease is likely to respond to treatment as well (1). Antigen-activated CD4+ T cells stimulate monocytes, macrophages, and synovial fibroblasts to produce the cytokines interleukin-1, interleukin-6, and TNF-α and to secrete matrix metalloproteinases through cell-surface signaling by means of CD69 and CD118 as well as through the release of soluble mediators such as interferon-γ and interleukin-17. Interleukin-1, interleukin-6, and TNF-α are the key cytokines that drive inflammation in rheumatoid arthritis. Activated CD4+ T cells also stimulate B cells through cell-surface contact and through the binding of \( \alpha \beta \) integrin, CD154 (CD40 ligand), and CD28, to produce immunoglobulins, including rheumatoid factor.

Patients with RA are considered to be at nutritional risk for many reasons. One cause of poor nutritional status in this patient population is thought to be the result of the weight loss and cachexia linked to cytokine production (2). In patients experiencing chronic inflammation, the production of cytokines, such as interleukin-1 and tumor necrosis factor, increases the resting metabolic rate and protein breakdown. The patient is then faced with the challenge of increasing both calorie and protein intake to meet the nutritional requirements of the increased metabolic rate. This is frequently difficult partly because of the pain and swelling associated with RA, which make food preparation and purchasing difficult for those who live alone or have limited resources. In the Indian subcontinent, malnutrition and anemia, weakness and an emaciated look with arthritis is a quite typical presentation in case of persons reporting to state government hospitals for free treatment, in the rural and semi-urban areas.

Blood transfusions are widely used as a rapid and effective therapeutic intervention. Transfusions are particularly helpful in the context of either severe anemia (in which the hemoglobin is less than 8.0 g per deciliter) or life-threatening anemia (in which the hemoglobin is less than 6.5 g per deciliter), particularly when the condition is aggravated by complications that involve bleeding. Blood transfusions are widely used as a rapid and effective therapeutic intervention and have been associated with increased survival rates in anemic patients.

My team of doctors has been successfully transfusing placental cord whole blood, which is rich in fetal hemoglobin content as well as cytokine and growth factors, as an alternative emergency source of blood transfusion in the background of anemia and emaciation of any etiology.
The placenta, or the after birth, is discarded routinely everywhere in the world (in India alone, there are more than 20 million placentas produced as afterbirth every year), and is actually a cause of environmental pollution in many parts of the developing world because it attracts natural scavengers and spreads infection, unless aseptically treated or incinerated. The centers of excellence in the western developed world have been working on the use of a tiny microscopical fraction of cord blood, i.e., CD34 stem cells only (.01 percent of the nucleated cells of the placental blood). Whether fetal hemoglobin rich placental umbilical cord whole blood (which has the potential to carry more oxygen to the tissue Vol/Vol than adult blood because of its fetal hemoglobin component, if collected aseptically after the birth of a healthy newborn at or near term), could be an emergency and safe substitute for adult whole blood in case of rheumatoid arthritis victims with hemoglobin concentration of less than 8 gm per deciliter, who cannot afford to buy erythropoietin injections or even arrange fresh packed cell for transfusion for them, was the main idea behind the present study. We received a grant (1999-2002) from the Department of Science and Technology, Government of West Bengal.

Materials and Methods

The problem of treatment of rheumatoid arthritis is somewhat different in developing countries, due to the poor socio-economic and educational backgrounds of the majority of patients. Here, for the most part, non-compliance with the suggested drug starts as soon as there is some relief. We frequently come across poor patients with intractable pain due to the progression of rheumatoid arthritis, with the involvement of inflammatory and neuropathic components of the disease. The ultimate goal in the therapy of rheumatoid arthritis is primarily reduction and relief of the pain and inflammation, and secondarily, maintenance of the functions and protection of the articular structures and systemic involvement.

We followed the American College of Rheumatology’s (ACR) revised criteria for inclusion of rheumatoid arthritis patients in the present study who had anemia (8gm/dl or low) in addition (3). While being well meaning and helpful in treatment guidance, strictly speaking, the criteria are not optimal in distinguishing early rheumatoid arthritis from undifferentiated polyarthritis and systemic lupus erythematosus. As per the suggestions of the ACR, one to three years of the disease process is considered as early disease in this government hospital based study conducted in Calcutta (India), where most poor patients are admitted in a free bed to receive free treatment.

The patients in this study included marginalized persons, i.e., homeless people, alcoholics, migrants, drug abusers, landless laborers and the poor from any strata of society. We enrolled patients from our hospital who were suffering from anemia, rheumatoid arthritis, emaciation and who could not buy erythropoietin or arrange for fresh whole blood or concentrated RBC, for cord blood transfusion. All enrolled patients gave proper informed consent and the institution based ethical committee approved each case.

Seventy eight units of human placental umbilical cord blood were collected from consenting mothers aseptically after lower uterine Cesarean section under general or regional anesthesia and the same was transfused (42ml -136 ml mean 80.6 ml+3.6 ml SD, median 82.4ml, mean packed cell volume 48.2 + 2.1 SD, mean hemoglobin concentration 16.4 Gm
percent + 1.5 Gm percent SD) from 1st April 1999 to April 2005 to 28 informed, consenting patients with advanced rheumatoid arthritis who had plasma hemoglobin 8gm per deciliter or less. In case of gross prematurely or dysmaturity, or if the projected weight of the fetus was less than 2 kg, or if there was any specific disease affecting the mother like hepatitis or HIV, etc., the cord blood collection was abandoned. Cord blood was collected from only informed, healthy mothers with their consent after the birth of their healthy babies. The collection process started only after the baby was safely removed from the operation field and the anesthetist verified the stable physical condition of the mother. It was only then that the obstetrician took the decision to proceed with the umbilical cord blood collection. Immediately, the cord was disinfected by spirit/Betadine solution at the site of the proposed puncture of the umbilical vein and a 16 g needle was attached to a standard pediatric collection bag (containing 14ml anticoagulant citrate phosphate dextrose adenine solution), which was used for the purpose of collection. When the collection was complete, the blood bag tubing was closed, sealed, and stored at 1- 4 degree centigrade, after putting necessary identification markings. Another sample of the cord blood collected from the placenta was immediately tested for blood group (Rh and ABO), HIV (1 and 2), hepatitis B and C, VDRL, malaria, fungus and bacterial study, as per standard blood transfusion protocol, on which we have reported earlier (4,5,6,7). The collected cord whole blood was transfused immediately or at the most, within three days of collection to a patient with anaemia (vide Fig 1), after grouping, cross-matching and following the standard adult blood transfusion WHO guideline for screening and transfusion, strictly adhering to the institutional ethical committee’s instructions and the patient consent protocol. Pre-transfusion, and three days after the transfusion, blood was drawn from the consenting patients for peripheral blood hematopoietic stem cell estimation (CD34) by flow analysis cytometry as per standard protocol at Ranbaxy Laboratory.

Figure 1: showing the photograph of an anemic patient with arthritic background receiving cord blood transfusion
Result and discussion

In the present series 28 patients with arthritis volunteered and were included (as per the American College of Rheumatology (ACR) revised criteria for inclusion) for the cord blood transfusion protocol to combat anemia. The background pre transfusion hemoglobin varied from 5.6 to 7.9 gm per deciliter. Each patient received 2 units to 6 units of cord blood within a span of 15 days depending on the availability and need. The age of the patients varied from 4 years to 62 years. 18 of them were female and the rest (10 patients) were male. O (Rh+) was the commonest blood group (11 cases), followed by A(Rh+) in 7 cases. Blood group B (Rh +) was present in 6 cases and the remaining 4 cases belonged to the AB (Rh +) group. A total of 78 units of freshly collected cord blood were transfused to the arthritic volunteers. The blood was transfused as soon as it was collected from consenting mothers and the screening, grouping and cross matching was completed. The volume of cord blood varied from 42ml -136 ml mean 80.6 ml+3.6 ml SD, median 82.4ml, mean packed cell volume 48.2 + 2.1 SD, mean hemoglobin concentration 16.4 Gm percent + 1.5 Gm percent SD. The study, which began in April 1999, was followed up till April 2005. Not a single post-transfusion patient suffered from any immediate or late complication of blood transfusion, i.e., immunological or non-immunological reaction. There was a rise in body weight of 3 to 5 lbs in 75 percent of the patients. A sense of well being, both subjective and objective, as well as an improvement in appetite was present in all the patients.

We did peripheral blood CD34 study by flow analysis cytometry before cord blood transfusion and 72 hour after transfusion and it was repeated again after three months. Three days after completion of the transfusion, the peripheral blood hematopoietic stem cell (CD34) estimation revealed a rise from the pre transfusion base level (.09 percent), varying from 2.03 to 23 percent. The flow analysis report of a case is shown 72 hours after two units of cord blood were transfused in Fig. 2. The report shows the increase of peripheral blood CD34 to 5.3 percent. This returned to base level in most cases at the three monthly CD34 re-estimation, without provoking any clinical graft vs host reaction in any of the patients.

Anemia in rheumatoid arthritis is a very complex phenomenon of cytokine interregulation and belongs to a specific sub group of anemia known as anemia in chronic disease and is the second most prevalent cause of anemia. The first important cause is the dietary iron deficiency. In case of anemia in chronic disease there is acute or chronic immune activation of specific cytokine system, which helps in shifting of the iron from its normal route. The condition has also been termed as "anemia of inflammation" (8). This condition is immune driven; with the cytokines and cells of the reticuloendothelial system induce changes in iron homeostasis, the proliferation of erythroid progenitor cells, the production of erythropoietin, and the life span of red cells, all of which contribute to the pathogenesis of anemia. Erythropoiesis can be affected by disease underlying anemia of chronic disease 9-10; it can be due to pro-inflammatory cytokines and free radicals that damage erythroid progenitor cells. Bleeding episodes, vitamin deficiencies (e.g., of cobalamin and folic acid), hypersplenism, autoimmune hemolysis, may also contribute to the anemic process affecting diseases like rheumatoid arthritis. Freshly collected cord blood, rich in hemoglobin and growth factors, may have a positive impact on the anemia of chronic disease.

Figure 2: The flow analysis cytometry report of the peripheral blood CD34 level (5.03 percent in the peripheral blood) 72 hours after the ABO/Rh group cross matched cord blood transfusion.
Rheumatoid arthritis is the commonest form of inflammatory arthritis and it affects about 1 to 3 percent of the population in the western hemisphere. The clinical presentation is heterogeneous with a wide variation in age at onset, degree of joint involvement, and severity. Most patients with aggressive disease will become clinically disabled within 20 years. Furthermore, in patients with severe disease or extra-articular symptoms, mortality is equal to that for patients with triple artery coronary artery disease or stage IV Hodgkin's lymphoma (9). Although rheumatoid arthritis predominantly affects peripheral joints, discovertebral joints of the cervical spine are often affected. Because arthritis is more prevalent in overweight adults, it accounts for 7.4 percent of admissions (10). The long-term prognosis in this disease is very poor (11). Recent advances in rheumatoid arthritis provide an insight into new therapeutic updates. However, these therapies appear to be suited for well-to-do patients with medical insurance, especially those who live in western, developed countries. The problem of rheumatoid arthritis is compounded in countries with inadequate resources, as a result of economic constraints, malnutrition, and a limited number of specialists (12). Most affected patients initially go to a primary care physician in developing countries like India. If the patient's condition is mixed with compounding complications such as bone destruction, severe pain, and the development of fibrous or bony ankylosis (13), or the progress of vasculitis, activation of sub clinical tuberculosis, restrictive lung disease, renal parenchyma's disease, hypothyroidism, altered glucose tolerance, frank diabetes, and cardiomyopathy, the scenario which emerges is typical of a government hospital arthritis patient in Calcutta (India), and its probable presentation.

Anemia is one of the essential co morbidity frequently associated with the arthritic process, more so in under resourced patients where the cost of treatment is unaffordable for many. As a result, many patients frequently discontinue the treatment with slight remission. Diet may play role in the management of RA, particularly in alleviating the symptoms of the disease, combating the side effects
of therapy and reducing the risk of complications. Proper antioxidant nutrients (Vitamin A, Vitamin C, selenium) may provide an important defense against increased oxidant stress and a supplementation of folate and Vitamin B12, in patients treated with methotrexate (MTX), can reduce the incidence of side effects and offset the elevation in plasma homocysteine, which is frequent in these patients. Calcium and vitamin D, in patients treated with corticosteroids, can reduce bone loss, while a simple supplementation with iron may not always prevent anaemia. But such a balanced diet containing all the micronutrients and protein is not affordable to under-resourced and marginalized people who report to government hospitals for free treatment. The cause behind anemia in arthritis is also not so simple that a properly balanced diet can fully alleviate the problem. Neither iron or folic acid, nor B12 supplement can effectively reverse the condition of anemia in arthritis.

Recent studies have given us some clues on the causation and perpetuation of anemia in arthritis and other chronic diseases. Hepcidin, an iron-regulated acute-phase protein that is composed of 25 amino acids, has helped to shed light on the relationship of the immune response to iron homeostasis and anemia of chronic disease. Hepcidin expression is induced by lipopolysaccharide and interleukin-6 and is inhibited by TNF-α (14). Transgenic or constitutive over-expression of hepcidin results in severe iron-deficiency anemia in mice (15).

Another option to tackle anemia is to inject erythropoietin provided there is no dearth of iron or B12 store. However, there is little data currently available on the possible effects (of erythropoietin?) on the course of the underlying disease, particularly since epoetin ? (Or erythropoietin?) can exert additional biologic effects, including interference with the signal-transduction cascade of cytokines (16).

A hallmark of anemia of chronic disease is the development of disturbances of iron homeostasis, with increased uptake and retention of iron within the cells of the reticuloendothelial system. This leads to a diversion of iron from the circulation into storage sites of the reticuloendothelial system, subsequent limitation of the availability of iron for erythroid progenitor cells, and iron-restricted erythropoiesis. In mice that are injected with the pro-inflammatory cytokines interleukin-1 and tumor necrosis factor α (TNF-α) (17), both hypoferremia and anemia develop; this combination of conditions has been linked to cytokine-inducible synthesis of ferritin, the major protein associated with iron storage, by macrophages and hepatocytes (18). In chronic inflammation, the acquisition of iron by macrophages most prominently takes place through erythrophagocytosis (19) and the transmembrane import of ferrous iron by the protein divalent metal transporter 1 (DMT1) (20). Interferon-γ, lipopolysaccharide, and TNF-α up-regulate the expression of DMT1, with an increased uptake of iron into activated macrophages. These pro-inflammatory stimuli also induce the retention of iron in macrophages by down-regulating the expression of ferroportin, thus blocking the release of iron from these cells. Ferroportin is a transmembrane exporter of iron, a process that is believed to be responsible for the transfer of absorbed ferrous iron from the duodenal site (21).

Calcium and vitamin D, in patients treated with corticosteroids, can reduce bone loss, while a simple supplementation with iron may not always prevent anaemia. But such a balanced diet containing all the micronutrients and protein is not affordable to under-resourced and marginalized people who report to government hospitals for free treatment. The cause behind anemia in arthritis is also not so simple that a properly balanced diet can fully alleviate the problem. Neither iron or folic acid, nor B12 supplement can effectively reverse the condition of anemia in arthritis.

In the present series, the patients enrolled for cord blood transfusion were not able to afford injections of erythropoietin or fresh packed cell to combat their anemia in the background of chronic arthritis. In India more than 20 million registered births take place per year, and there is potentially an abundant supply of placental cord blood. In our hospital, we used the freshly available placental blood, rich with cytokine and growth factors, from our own in patient department, and it worked well. The question is why this is so? Why was there not a single case of immunological or non immunological reaction and why did the patients who received the cord blood gain weight? The answer may be found in the hypoantigenicity of the fetal system. Our group of doctors have been working on fetal cell /tissue transplant in adult
degenerative diseases and we have published the reports of our experience from time to time (26,27,28,29,30). The basic reason for non-rejection of the conceptus is the fact that pregnancy and neoplasm are two outstanding examples of natural tolerance to homograft. In order to avoid maternal HLA systems’ recognition, there is non cytotoxic antibody inside the placenta apart from hypoantigenic fetal cells. At or near term, however, a slow bi-directional traffic of cells at the fetal-maternal interface slowly develops. Moreover, fetal progenitor cells have been found to persist in maternal peripheral blood for decades after childbirth. Progenitor cells can differentiate into mature immune-competent cells. Chimerism is used to indicate a body that contains cell populations derived from different individuals; microchimerism indicates low level of DNA of presumed fetal origin, can be detected in the mother circulation decades after delivery and is referred to as fetal microchimerism (FM) (31). Lymphohemopoietic cytokines are now recognized to be central participants in the cellular communication events underlying the complex and dynamic remodeling processes required to accommodate the semiallogeneic conceptus during mammalian reproduction. Cytokines are identified to be of particular importance in mediating communications between the conceptus and maternal cells, particularly the uterine epithelium and infiltrating leukocytes, both prior to implantation and as the placenta develops.

What is intriguing is the rise of peripheral blood CD34 level after cord blood transfusion in case of non pregnant recipient, as seen in the flow analysis cytometry report. The reason for the transient rise of hematopoietic stem cells as seen in the peripheral blood in HLA randomized recipients without any immunosuppressive support and without provoking clinical graft vs host reaction, still remains a mystery.

However, we can venture some probable explanations. The placenta has a unique microenvironment and its sensitization impact on cord blood cells may have a role in transient transplantation impact on the host system. One very important factor, apart from intrinsic differences, is the fact that HSC (hematopoietic stem cell) in UCBC (umbilical cord blood cell) have had a different set of microenvironmental exposures compared to those of adult marrow or PBSC (peripheral blood stem cell). An example of differences between sources are some of the observed changes in HSC cell cycle status, gene expression and the adhesive and invasive properties induced by mobilization procedures used to generate PBSC, e.g., G-CSF (granulocyte colony stimulation factor). The placenta is a complex organ that regulates maternal-fetal interactions (32) . This placental environmental exposure of cord blood Male DNA, of presumed fetal origin, can be detected in the host either due to drugs, chronic nature of the disease, malnutrition with helminthiasis, or other associated factors like the impact of growth factors or selective cytokine impact of the cord blood on the bone marrow of the recipient, may help in the transient rise in the CD34 in the host. Our preliminary bone marrow study also suggested a positive impact on the host bone marrow cellularity in those patients.

Conclusion:

Rheumatoid arthritis is the commonest disorder of connective tissues and is an important cause of disability, morbidity, and mortality, and life expectancy is reduced by four years in men and by 10 years in women. The overall incidence of the female: male involvement is 3:1. It is associated with serious infections, vasculitis (e.g., ulcers, mononeuritis), anemia, thrombocytopenia, and lymphadenopathy. Extra-articular manifestations of rheumatoid arthritis include vasculitis, pulmonary involvement with alveolitis, eventually leading to varying degree of the fibrosis of the lung. Cardiac involvement includes pericarditis, which is common, apart from conduction defect, mitral valve disease and varying degrees of cardiomyopathy. Cutaneous involvement implies vasculitis, palmar erythema and pyoderma gangrenosum. Little is known about the primary cause of RA. Although the primacy of T-cell-related events early in the disease remains debated, strong evidence indicates that autoantigen recognition by specific T cells is crucial to the pathophysiolo of rheumatoid synovitis (33). The massive influx of T cells into the arthritic joint is accompanied by the energization of over 90 percent of T cells in this compartment – which further substantiates the concept of the RA attractor within the self-regulating immune system. Thereby, the RA-attracted immune system is not able to completely down regulate the inflammation and the local tissue damage/repair. Thus, the immune system is permanently stimulated and suddenly by chance shifts to a stable state different from the healthy system—reaching the wide fields of rheumatoid arthritis which in itself is self-sustaining as the
healthy state before disease onset (34). Anemia in chronic diseases like arthritis is a complex process
and its dysregulation in inflammation induced cytokine interplay is poorly understood.
The underresourced world has underresourced patients and doctors have very little options or for that
matter, material resources. This stimulates clinical research for alternatives for those who cannot
afford to buy the prescribed medicines and the support. We have seen that cord blood collected
aseptically at birth which is rich in cytokine and growth factors along with high fetal hemoglobin
plasma, can be used in the case of those desperately ill patients who cannot buy or arrange
erthropoietin or fresh blood for their transfusion requirements. The rise in CD34 after cord blood
transfusion is an unique phenomenon in HLA randomized recipients without specific
immunosuppressive support which we are studying further for its cell therapy and bone marrow
rejuvenating role.

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Introduction: Leprosy is a chronic infectious disease, caused by acid-fast mycobacterium known as mycobacteria leprae. It affects the skin, and the peripheral nerves and can also affect the eye, the upper respiratory tract, etc. In the year 1873, the famous Norwegian physician, Gerhard Henrik Armauer Hansen, first identified the organism. The name leprosy came from the Latin word lepros, which means defilement. This disease is documented since antiquity. Leprosy was considered a divine curse for sin in the Old Testament and is also documented in Hindu and Buddhist texts as the result of Karma, i.e., reaction for committing injustice or crimes against man. Earlier, in a lay mind, the disease was deemed as incurable due to its slow spread, and because it caused severe deformity, disability and social stigmatization, as a result of different myths behind the disease. Leprosy was endemic in Western Europe since the medieval period. This was eliminated in Scandinavian countries as recently as the early twentieth century, as a result of improved socioeconomic index; the people were provided with better housing, nutrition, clean water supply and there was an overall improvement in living standards.

In 1991, the World Health Organization (WHO) and its member states committed themselves to eliminate leprosy as a public health problem by the year 2000. While leprosy is still a major problem in most developing countries, India and Brazil are among the top six countries in the world with a prevalence rate of 4.3 to 4.5 per 10,000 persons. India alone accounts for the 78 percent of the 690,830 newly detected cases in 2001(1,2,3).

The major goals of the leprosy control programme are early detection, proper treatment, and adequate care for prevention of disabilities and rehabilitation. There are several effective chemotherapeutic agents against M. leprae, of which Dapsone (diaphenylsulfone, DDS), Rifampicin, Clofazimine, Ofloxacin and Minocycline constitute the backbone of the multidrug therapy as
recommended by the WHO. Gastrointestinal toxicity and skin discoloration are the major side effects for long-term treatment with clofazimine, and rifampicin is known for its hepatotoxicity. But the most important side effect, which is prominent, is the hematological problems with Dapsone(4). The common hematological side effects with Dapsone therapy are hemolytic anaemia, methaemoglobinaemia, reticulocytosis, and reduction of cell resistance as seen in osmotic fragility studies. These effects are much more pronounced in Lepromatous leprosy patient with blunted erythropoietin response with low serum iron and mildly raised serum ferritin concentration. The problem of Dapsone therapy is complicated further in endemic areas where, because of low nutrition, malaria and intestinal parasitism, the hemoglobin concentration is already compromised.

In order to combat anaemia in leprosy patients, with varying degrees of refractoriness due to drug on the disease or the host reaction (5,6,7,8,9,10), Dharmendra (11), a noted leprosy specialist from India, suggested many years back, blood transfusion from other consenting leprosy patients. In India, the placenta is a readily available source of fresh whole blood, which is rich in fetal hemoglobin, and which can carry more oxygen than adult hemoglobin. This placenta or the after birth is discarded routinely everywhere in the world and (in India alone, there are more than 20 million placentas produced as afterbirth every year) is actually a cause of environmental pollution in many parts of the developing world because it attracts natural scavengers and spreads infection, unless aseptically treated or incinerated. The centers of excellence in the western developed world have been working on the use of a tiny microscopical fraction of cord blood, i.e., CD 34 stem cells only (.01 percent of the nucleated cells of the placental blood). My team of doctors has been successfully transfusing placental cord whole blood, which is rich in fetal hemoglobin content, as an alternative emergency source of blood transfusion in the background of anaemia and emaciation of any etiology.(12,13).

Whether fetal hemoglobin rich placental umbilical cord whole blood (which has the potential to carry more oxygen to the tissue Vol/Vol than adult blood because of its fetal hemoglobin component, if collected aseptically after the birth of a healthy newborn at or near term), could be an emergency and safe substitute for adult whole blood in case of leprosy victims with hemoglobin concentration of less than 8 gm percent, was the main idea behind the present study. We received a no objection clearance from the Department of Health and Family Welfare, Govt. of West Bengal, for this study.

Material and Methods: 74 units of human placental umbilical cord blood were collected from consenting mothers aseptically after lower uterine Caesarean section under general or regional anesthesia. If there was gross prematurity or dysmaturity or the projected weight of the fetus was less than 2 kg, or there was any specific disease of the mother like hepatitis or HIV, etc., the cord blood collection was abandoned. Cord blood was collected from only informed, healthy mothers with their consent after the birth of their healthy babies. The collection process started only after the baby was safely removed from the operation field and the anesthetist verified the stable physical condition of the mother. It was only then that the obstetrician took the decision to proceed with the umbilical cord blood collection. Immediately, the cord was disinfected by spirit/Betadine solution at the site of the proposed puncture of the umbilical vein and a 16 g needle was attached to a standard pediatric collection bag (containing 14ml anticoagulant citrate phosphate dextrose adenine solution), which was used for the purpose of collection. When the collection was complete, the blood bag tubing was
closed, sealed, and stored at 1-4 degree centigrade, after putting necessary identification markings. Another sample of the cord blood collected from the placenta was immediately tested for blood group (Rh and ABO), HIV (1 and 2), hepatitis B and C, VDRL, malaria as per standard blood transfusion protocol, on which we have reported earlier (14). The collected cord whole blood was transfused within 3 days of collection to a leprosy victim with anaemia, after grouping, cross matching and following the standard adult blood transfusion WHO guideline for transfusion and strictly adhering to the institutional ethical committee guidelines and the patient consent protocol. Pre transfusion and seven days after the transfusion blood was drawn from the consenting patients for peripheral blood hematopoietic stem cell estimation (CD34) by flow analysis cytometry as per standard protocol at Ranbaxy laboratory which was repeated after 3 months for comparison.

Result and Analysis: Leprosy is almost exclusively a disease of the developing world with 80 percent of its victims residing in India, China, Indonesia, Myanmar, Brazil, Nigeria, etc. The disease is practically absent in USA (less than 200 cases detected from outsiders per year). Poverty, rural background and its impact on socioeconomic, educational and nutritional status are very important for the long incubation and transmission of the disease. We are a group of investigators in Calcutta (India) working on the problem of anaemia among leprosy victims undergoing treatment. Varying degrees of anaemia are very much prevalent in patients of Leprosy, which could be due to background malnutrition, coexistant diseases like helminthiasis or drug impact on the immune system, (including the bone marrow), poor red cell survival, and rarely Glucose –6-phosphate dehydrogenase deficiency (Dapsone therapy). We have so far treated 16 cases (15 male+ 1 female, Age 12-72 yrs, mean 48.4 yrs). Five cases were of the pausibacillary type (PB) and 11 cases were of the multibacillary type (MB). The clinical spectrum varied widely from the tuberculoid to the lepromatous type and one patient presented with gangrene of the leg preceding auto amputation and was infested with maggots (vide photograph of Figure 1). MB patients received Rifampicin 600mg/once monthly and Clofazimin 200mg initially, followed by 50 mg daily, along with Dapsone 100mg daily for 12 months uninterrupted. PB patients receive 600mg Rifampicin once monthly along with Dapsone 100mg daily for 6 months. In the present series we collected 74 units of placental umbilical cord whole blood after lower uterine caesarean section (LUCS), from consenting mothers and transfused these (Vol 52-142 ml mean 83ml and 14 ml S.D) to leprosy patients with anaemia within 3 days of collection (with the blood being kept in a refrigerator earlier). We followed the standard safe transfusion protocol as per WHO guidelines, and transfused 2 units to 8 units of placental blood to each patient without encountering any immunological to non immunological reactions so far. Immediate reactions due to transfusion viz.,fever, chill and rigor, flank pain, back pain, blood in urine, fainting or dizziness was not seen in any of the cases. Even late reactions like mild or progressive renal complications were not encountered. Feto- maternal cell traffic has been implicated for the cause of scleroderma in mothers in case of male babies. In the present series, we did not see any such rare and unusual complication due to neonatal blood transfusion in the adult system, excepting one unusual reaction, on which we will now report.

In some leprosy patients, seven days after the completion of the cord blood transfusion, the flow analysis cytometry study showed a rise (pre cord blood transfusion peripheral blood CD34 normal range is up to .09 percent) of peripheral blood CD34 level, from the pretransfusion level ,varying from 3.6 percent to 16.2 percent, in 75 percent of the cases (A case where the FACS showed 5.65 percent peripheral blood CD34 is shown in figure 2). This effect became normal after three months. Questions may be raised as to why there was peripheralisation of the CD34 cell? There was no clinical sign of graft vs host reaction in any of the patients. There was no growth factor or any other bone marrow stimulating or suppressing drug utilized during the transfusion protocol for the cord blood.
Figure 1: Photograph showing an infected gangrene and ulceration which was studded with maggots, in the background of leprosy.
Discussion:
The possible reason behind the transient rise of hematopoietic stem cells as seen in the peripheral blood in HLA randomized recipients without any immunosuppressive support and without provoking clinical graft vs host reaction, still remains a mystery. However, probable explanations may be: the Placenta has a unique microenvironment and its sensitization impact on cord blood cells may have a role in UCBT (umbilical cord blood transplantation). Besides intrinsic differences, HSC (Hematopoietic stem cell) in UCBC (umbilical cord blood cell) have had a different set of microenvironmental
exposures compared to those of adult marrow or PBSC (Peripheral blood stem cell). All HSC sources are influenced by the microenvironment from which they are derived. An example of differences between sources are some of the observed changes in HSC cell cycle status, gene expression and adhesive and invasive properties induced by mobilization procedures used to generate PBSC, e.g., G-CSF. The placenta is a complex organ that regulates maternal-fetal interactions. Many cytokines that can influence lymphohematopoietic development, e.g., G-CSF (Granulocyte colony stimulation factor), c-kit ligand (stem cell factor [SCF]), GM-CSF (Granulocyte macrophage colony stimulated factor), IL-15 (Interleukin 15), and others, are produced by the placenta. Production of G-CSF by the placenta may be especially relevant to UCBT. G-CSF is produced both by the maternal decidua and the fetal chorionic villi and enters the fetal circulation by a process that does not require a functional G-CSF receptor. G-CSF from the mother probably does not enter the fetal circulation as administration of recombinant human G-CSF (rhG-CSF) to pregnant macaques did not result in detectable rhG-CSF in the fetuses. The function of placental G-CSF production is unknown; however, it may serve as an immunoregulator that protects the mother and fetus from each other’s allogeneic immune systems. G-CSF inhibits the ability of placental mononuclear cells to mediate cytotoxicity against allogeneic targets including choriocarcinoma cells.

In fine, though precisely not clear as yet, the functional hypoantigenicity of the cord blood antigen with its complex cytokine interaction may have a role in immune selective masking in leprosy, i.e., immune mosaicism, in those anemic patients with leprosy either due to Dapsone like drugs, disease, nutrition or hemlinthisis, or other associated factors like the impact of growth factors or selective cytokine impact of the cord blood on the bone marrow of the recipient. All the patients irrespective of their background tolerated the procedure well and there was a sense of well being in most of the cases.

Our group of researchers is working on the problem of fetal cell or tissue transplant in the adult system. We are also working on the use of umbilical cord whole blood transfusion as an alternative to adult whole blood transfusion from the pediatrics to the geriatric age group in different indications since 1st April 1999. We received a research grant from the Dept of Science and Technology of the Govt of West Bengal, India, and have published our reports from time to time (16,17,18,19,20). We are of the opinion that the growth factor and cytokine filled cord blood collected aseptically from the placenta of the consenting mother after the birth of a healthy baby, has all the potential of an effective therapeutic adjuvant for Leprosy patients with anemia in the underprivileged world. Cord blood is protected from infection as a result of nature’s finest biological sieve, i.e., the placenta, and contains 60-80 percent fetal hemoglobin (which can carry 60 percent more hemoglobin than adult hemoglobin), and moreover, has a high WBC and platelet content, is hypoantigenic in nature, and has an altered metabolic profile. It may also have the potential (7), which we are studying at present, to convert TH2 responses (Lepromatous) to TH1 response (Tuberculoid), due to its rich cytokine and growth factor content, which may have a role in immune response modification.

Conclusion: UCB is now accepted as an alternative source for hematopoietic stem cells for transplantation especially in children. Unrelated UCB offers many practical advantages as an alternative source of stem cells, including: (1) easy availability without risk for mothers and donors; (2) less possibility of infection, particularly cytomegalovirus; (3) documented reduced risk of GVHD, with easy HLA matching criteria for donor-recipient selection and (4) UCB banks have been established for related and unrelated UCBT with about 100,000 units currently available (21.)

In this preliminary communication and follow up for 6 years, we have seen that cord blood transfusion is safe in leprosy patients with anemia; however, the unique phenomenon of transient spontaneous transplant impact of the CD34 cells, as seen in the peripheral blood after cord blood transfusion in HLA randomized recipients, without the support of the immunosuppressives, without provoking clinical graft vs host reaction, which has never been cited earlier in published medical literature.
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Introduction:

In the animal kingdom swallowing the afterbirth by the mother is a general norm. Nature appears to have provided this precious wisdom to some of its creatures. Even herbivorous animals swallow the placenta after the birth of their babies (for example, the cow). But humans do not seem to know how to use this precious afterbirth, which has protected and nurtured the baby for so long in the womb. Of late, however, since 1989, (Ref 1, 2) Through evolution, the oxygen free carrying pigment in invertebrates became intracellular for better tissue perfusion in response to growth and metabolic demands from lower vertebrates to higher vertebrates. By incorporating the hemoglobin inside the RBC rather than carrying oxygen in plasma alone, the evolutionary process increased by about 100 times the oxygen carrying capacity of higher vertebrates. If hemoglobin is extracellular and intravascular, it may exert 5 times more osmotic pressure than plasma protein. As a result, water will be drawn from the tissue space and load the intravascular compartment. By inclusion of the hemoglobin inside the cell, the viscosity of the blood remains low, water is not drawn from the tissue space and the flow of blood with a large protein content is made possible. In addition, RBC membranes contain enzymes that protect the hemoglobin from degradation and allow it to work for three months at a stretch. A lack of enzymes in the membrane free hemoglobin leaves it at risk from oxidative damage by the haem molecule (Ref 3) Furthermore, free hemoglobin is always subject to oxidative denaturation apart from acting as a trigger for hypertensive episodes.

In the global search for a suitable hemoglobin based oxygen carrier from human RBC to bovine RBC, or its chemically or genetically modified form, or even from sea creatures (Arenicola Merina), i.e., sea worm, the hemoglobin has been extracted (Ref 4) for its potential human use. Animal hemoglobin can trigger allergic reactions and can even damage the kidneys.

Adult hemoglobin consists of 2 alpha and 2 beta polypeptide chains, each bound to a haeme group, capable of binding with one molecule of O2 (1 Gm hemoglobin binds with 1.39 ml of oxygen). Therefore, 14 gm percent of adult hemoglobin can carry, on an average, 19.46 ml of oxygen. Cord blood at term carries on an average 16.8 Gm percent hemoglobin (Ref 5) of which 20 percent belongs to the adult hemoglobin type (3.36 gms) and 80 percent belongs to the fetal hemoglobin type (13.44gms). The concentration of the fetal hemoglobin may increase further depending on fetal stress, maturity and several other fetomaternal factors. Fetal hemoglobin has the potentiality to carry upto 50 percent more oxygen than adult hemoglobin (Ref 6), i.e., 1Gm of fetal hemoglobin may carry upto 2.08 ml of oxygen. If we simply calculate theoretically the oxygen carrying potentiality of 100ml of cord blood taking into account of its 80 percent fetal hemoglobin component (2.08 ml O2 carrying capacity per gm of fetal hemoglobin) and 20 percent adult hemoglobin component (1.39ml O2 carrying capacity per gm of adult hemoglobin), it would be around 32.62 ml of O2 carrying capacity, which is a 67.62 percent additional oxygen capacity of the adult blood (19.46 ml Oxygen/100 ml). There are several factors which modify the O2 binding affinity, which includes, (a) concentration of hydrogen ion, (b) carbondioxide concentration in the blood, (c) body temperature, (d) 2-3 diphosphoglycerate concentration only, to name a few.

Material and Methods:

413 units of human placental umbilical cord blood was collected from consenting mothers aseptically after lower uterine Caesarean section under general or regional anaesthesia. If there was gross prematurity or dysmaturity or the projected weight of the fetus was less than 2 kgs., or there was any specific disease of the mother like hepatitis or HIV, etc., the cord blood collection was abandoned. Cord blood was collected from only informed, healthy mothers with their consent after the birth of their healthy babies. The collection process started only after the baby was safely removed from the operation field and the anaesthetist verified the stable physical condition of the mother. It was only then that the obstetrician took the decision to proceed with the umbilical cord blood collection. Immediately the cord was disinfected by spirit/Betadine solution at the site of the proposed puncture of the umbilical vein and a 16 g needle was attached to a standard pediatric collection bag (containing
14ml anticoagulant citrate phosphate dextrose adenine solution), which was used for the purpose of collection. A second bag was used if the collection exceeds or nears 100ml and a second prick was made at a proximal region after using a clamp at the first site of prick. The blood flows by gravity and generally within a minute 90 percent of the collection is over and within 2 minutes, in most of the cases, the blood flow ceases completely due to clot formation. In case of any confusion about the condition of the baby, the decision was immediately taken to preserve the blood in consultation with the paediatrician for future use by the baby, or stamped "Unsafe for transfusion", and no risk or chance whatsoever was taken for the eventual recipient of the blood.

When the collection was complete blood bag tubing was closed, sealed, and stored at 1-4 degree centigrade, after putting necessary identification markings. Another sample of the cord blood collected from the placenta was immediately tested for blood group (Rh and ABO), HIV(1 and 2), hepatitis B and C, VDRL, malaria as per standard blood transfusion protocol, which we reported earlier (7).

Osmotic fragility study with 45 percent NaCl (N=40) at 4°C centigrade, 35°C and 40°C with a time gap of 24 hours, 7 days and 14 days along with oxyhemoglobin (m mole/ml) and plasma hemoglobin (mg/ml) assessment in identical schedule showed that the cord blood was reasonably stable at room temperature. In case of any confusion/contamination, the culture was put aside for identification of the pathogen if any, through appropriate protocol, and the sample was stamped unfit for transfusion.

In the present series, the collection of the blood varied from 50ml -146 ml mean 86 m1+7.6 ml SD, median 80 ml, mean packed cell volume 48 + 4.1 SD, mean hemoglobin concentration 16.2 Gm percent + 1.8 Gm percent SD. After collection the blood was immediately preserved in the refrigerator and transfused within 72 hours of collection. Donation of the cord blood to the recipient followed the strict guidelines of the human ethical committee of the Hospital headed by an emeritus Professor of Medicine. As a rule, the volunteer who wishes to enroll for the cord blood transfusion programme, must have a hemoglobin count which is below 8 Gm percent. Patients with cancer or some critical illness were given a priority. Before the umbilical cord blood transfusion, a thorough clinical examination of the recipient was done, including the proper monitoring of the BP/Pulse/Respiration and other cardinal and presenting features. Then pre-transfusion, a little blood was drawn from the prospective recipient of cord blood for Blood grouping, Hb/ Tc/ Dc/ ESR/ Platelet/ Coombs test, C-Reactive protein, Urea, Creatinine, Bilirubin and other investigations as per the requirements of the case. For example, Hb electrophoresis was done in case of thalassemia, before and after the transfusion was undertaken to see the impact of transfusion. A little blood was redrawn from the same patient who received cord blood transfusion, after 24 hours, 72 hours, 7days, 1month, 2 months, 3 months and subsequently, clinical follow-up continued at the OPD from time to time, to study the effect of transfusion and adverse reactions if any.

Actual transfusion procedure started after necessary grouping and cross-matching of the specimens and checking the identity of the patient. The cord blood was transfused by a blood transfusion set containing a filter (230 um). For the initial 15 minutes or so the patient was carefully observed to see if there was any transfusion reaction. Thereafter, if all went well, the transfusion rate was increased till it was completed.

Result and Analysis

| Disease-wise Distribution of Patient |  |
| Cancer | Others |

53
Fig (1) depicts the disease-wise distribution of the patients. Out of 129 cases, 56.58 percent patients were suffering from cancer, and the rest, i.e., 43.42 percent, of the patients were suffering from other diseases.

Fig (2) narrates the sex-wise distribution of the 129 cases who were enrolled in the present trial. 41.86 percent participants were male and the rest (58.14 percent) participants were female.

Fig (3) shows the blood group distribution of the patients. In the present series, 24.03 percent belonged to blood group A+, 31.78 percent belonged to blood group B+, 13.95 percent patients belonged to blood group AB+, 28.68 percent patients belonged to blood group O+, .77 percent patients belonged to O- and B- groups each.
Fig (4) depicts the age group distribution of the patients. Of the 129 patients who enrolled for the present cord blood transfusion protocol, the majority belonged to the 10 – 60 years age group, i.e., 79.86 percent. Patients below 10 years of age constituted 2.32 percent cases. Patients above 60 years, made up 17.82 percent of the cases.

Study results on the stability of cord blood at temperature and time

**Mean Fragility (% hemolysis in 0.45% NaCl) with Standard deviation (N= 40)**

<table>
<thead>
<tr>
<th>Temp</th>
<th>24hr</th>
<th>48hr</th>
<th>7 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C</td>
<td>12.6 + 3.4</td>
<td>32.9 + 4.3</td>
<td>45.6 + 2.8</td>
<td>82.5 +4.6</td>
</tr>
<tr>
<td>35°C</td>
<td>16.8 + 2.7</td>
<td>20.4 + 4.3</td>
<td>53.5 + 3.9</td>
<td>100</td>
</tr>
<tr>
<td>40°C</td>
<td>45.0 + 6.4</td>
<td>77.5 + 3.8</td>
<td>92.6 + 4.8</td>
<td>100</td>
</tr>
</tbody>
</table>

**Mean Oxyhemoglobin (mmole/ml) with Standard deviation (N =34)**

<table>
<thead>
<tr>
<th>Temp</th>
<th>24hr</th>
<th>48hr</th>
<th>7 days</th>
<th>14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>4°C</td>
<td>0.32 +.12</td>
<td>0.31 +.14</td>
<td>0.27+.05</td>
<td>0.26+12</td>
</tr>
<tr>
<td>35°C</td>
<td>0.31+.06</td>
<td>0.28+.03</td>
<td>0.24+.06</td>
<td>-</td>
</tr>
<tr>
<td>40°C</td>
<td>0.16+</td>
<td>0.09+.01</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Mean Plasma hemoglobin (mg/ml) with Standard Deviation (N =36)**

<table>
<thead>
<tr>
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<th>48hr</th>
<th>7 days</th>
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<tbody>
<tr>
<td>4°C</td>
<td>6.08+.87</td>
<td>6.35+.78</td>
<td>7.04+.89</td>
<td>9.69+1.7</td>
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<tr>
<td>35°C</td>
<td>4.49+.54</td>
<td>7.65+.86</td>
<td>10.0+2.3</td>
<td>-</td>
</tr>
<tr>
<td>40°C</td>
<td>10.3+1.6</td>
<td>13.3+2.4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
In the present series we are presenting the data of 413 units of aseptically collected cord blood from consenting mothers undergoing LUCS from 1st April 1999. Transfusion services and effective follow up have been continued in the outpatient department to date. The blood was transfused to 129 informed consenting volunteers after the cases were passed through the institution based ethical committee. The list included 54 male and 75 female patients. The age of the patients varied from 2 years to 86 years. 73 patients (56.58 percent) were suffering from advanced cancer and 56 (43.42 percent) patients suffered from other diseases. Three pediatric patients were less than 10 years old and one patient was more than 80 years old. 22 patients (17.05 percent) were within 60-80 years. The majority of the patients (50.38 percent) were within the 40 to 60 years age group. B+ was the commonest blood group (51.78 percent), followed by O+ (28.68 percent), then A+ (24.03 percent), and lastly AB+ (13.95 percent). O- and B- blood was transfused to one patient each. 33 units (highest) of cord blood was transfused to a patient with advanced cancer with hemoptysis (10 units of cord blood was transfused at a time), followed by 23 units to a patient with aplastic anemia (9 units of cord blood at a time), 16 units in a case of transfusion dependent thalassemic syndrome (8 units of cord blood at a time), followed by 15 units to a patient suffering from stage IV cancer with metachronous metastasis (7 units of cord blood at a time). Among others, those who received more than three units at a time included one patient who was given a transfusion of 10 units of cord blood; 3 patients received 9 units each, 2 patients received 8 units each, 18 patients received 7 units each. In addition, 7 units and 7 patients received 5 units of umbilical cord whole blood transfusion each. All patients presenting with anemia (8 Gm hemoglobin or less) and distress, be it in the background of Ankylosing spondylitis, lupus erythematosus, rheumatoid arthritis, aplastic anemia, thalassemia major, to bleeding per rectum or hemoptysis due to malignancy, responded clinically with cord blood transfusion.

The blood volume of a term fetus is approximately 80 - 85 ml/kg (8). The placental vessel at term contains approximately 150 ml of blood (Ref 9). The cord blood contains three types of hemoglobin, HbF, HbA, HbA2, of which HbF constitutes the major fraction (50-85 percent) (Ref 10). HbA accounts for 15 - 40 percent of hemoglobin and HbA2 is present only in trace amounts at birth (Ref 11). HbF has a greater oxygen affinity than HbA (Ref 12). The oxygen tension at which the hemoglobin of the cord blood is 50 percent saturated is 19-20 mm of Hg, 6-8 mm Hg lower than that of normal adult blood. This shift to the left of the hemoglobin oxygen dissolution curve results from poor binding of the 2-3 diphosphoglycerate by HbF (Ref 13, 14). The potential complications of blood transfusion therapy can be grossly divided under two headings, immunological and non-immunological reactions (Ref 15). The immunological reactions are related to the stimulation of antibody production by the foreign alloantigens by the different components of transfusion, e.g, RBC, leucocytes, platelets and plasma proteins. Alloimmunizations may lead to immunological reactions in case of future stimulation by a similar antigen. The commonly encountered immunological reactions are haemolytic reactions due to red cell incompatibility. Febrile or pulmonary reactions are related to antigens of leucocytes and platelets. Allergic and anaphylactoid reactions are related to antibodies and it is only very rarely that we can see graft vs host reactions due to engraftment of the transfused lymphocytes in case of immunosuppression. The commonly encountered non-immunological reactions are because of physical or chemical properties of the transfused blood/blood products due to bacterial or viral contamination or the circulatory load.

During our experience of transfusion of 413 units of cord blood over the last five years, we have not encountered a single episode of immunological or non-immunological reaction so far. Fetal hemoglobin can carry more oxygen than the mothers blood and there is a potential advantage of the fetal hemoglobin (Bohr’s effect) by which it can carry more oxygen at low PCO2 than at high PCO2 (Ref 16). Another potent advantage of cord blood transfusion which has therapeutic implication, is the rich cytokine and growth factor filled plasma in the cord blood, which eventually has a positive effect on distressed and emaciated patients. On the basis of our experiences, we can say that cord blood transfusion is safe and can be used in hours of crisis from the pediatric to the geriatric age groups, as an alternative to adult whole blood transfusion, not as an inferior method of transfusion but as an effective supplementation of blood, which has no transfusion related hazards detected so far.

Discussion: Continuous supply of donated blood is vital for the practice of modern medicine, but due to an ever increasing worry over blood borne diseases like HIV, hepatitis or bovine spongiform encephalitis in certain areas, has fuelled the search for an alternative source for blood transfusion. Moreover, with the current global war against terrorism and other conflicts, the research to develop an ideal blood substitute has received a real boost. This has implications for not only the trauma and emergency surgeons, but the medical fraternity as a whole. Trauma surgeons, perhaps more than any
other health care provider, are the first to recognize the urgency of a real blood substitute without jeopardizing the safety aspect of such a transfusion. The current generation of blood substitutes are passing through US Food and Drug Administration (FDA) Phase –III clinical testing. These include RBC substitutes to provide the respiratory functions of hemoglobin, platelet substitutes and coagulation factors. (Ref 17) The most promising among the RBC substitutes, as mentioned earlier, is the hemoglobin extracted from the lysis of the RBC from human or bovine sources, or a chemically modified hemoglobin or a genetically engineered hemoglobin molecule. Although these hemoglobin based oxygen carriers have an intrinsic advantage of universal compatibility and storability at room temperature, because of the high cost involved, these would be simply unacceptable to the developing world in particular. Moreover, there are also specific problems of hypertensive impact, gastric irritability and unexplained deaths as reported in a trauma trial on the treatment of severe hemorrhagic shock. (Ref 18)

The other hemoglobin substitutes with lesser importance include perfluorocarbons, i.e., fluorine substituted with linear or cyclic carbon atoms with high oxygen carrying capacity, and liposome encapsulated hemoglobin (Ref 19).

Transfusion of adult blood is never a zero risk event anywhere in the world. Risks associated with adult blood transfusion include transmission of HIV (1 & 2), hepatitis B, C, A, G, Parovirus 19, specially in case of pregnancy, hemolytic anemia and immunocompromised background, apart from the possibility of transfusion of syphilis, kalaazar, malaria (in the developing world), unless the blood is thoroughly screened as per WHO and country specific guidelines. There are also problems of rare blood groups which are not screened normally but have the potentiality to trigger hemolytic reactions . There are many other reasons of transfusion specific acute or delayed immunological and non immunological reactions, contamination problems with platelet, RBC, etc. Very rarely, there could be an incidence of transfusion induced lung, liver or kidney injury. Lastly, there could also be problems due to immunomodulation. (Ref 20). Newly identified, but well known, potential risk factors include the possibility of the transmission of Creutzfeldt Jakob disease in its classical or varient form, even after leucodepletion (lymphocytes are possible source of transmission of infection) as reported in an editorial article in BMJ (Ref 21).

Attempts are being made by scientists and clinicians all over the world to make blood transfusions safer through stricter vigilance, emphasis on fewer transfusions and more conservation, preoperative autologous donation, stimulation of erythropoiesis, option for preoperative normovolumic hemodilution, attempts at intraoperative and post operative recovery of blood, inactivation of microbes in the platelet units, use of plasma with reduced viral activity and finally, the use of red cell substitutes (Ref 22). However, in spite of all these attempted maneuvers by clinicians, the risk of transfusions is not on the wane.

After our experience with 413 units of cord blood transfusion, we wish to affirm our faith in this safe transfusion protocol because we did not encounter a single case of immunological or non immunological reaction so far in any of our patients, even after the transfusion of 1 unit to 33 units (2838 ml on the basis of mean volume calculation) of cord blood to the same patient (with 10 units [mean 86x10 = 860 ml] of cord blood transfusion at a time) in different indications of blood transfusion from the paediatric to the geriatric age group (2 yrs to 86 yrs) in the common background of anemia with malignant or autoimmune or traumatic (surgical or non surgical), infective or congenital background disease (as in case of thalassemia). Our experience suggests that this placental cord blood transfusion could be an unique untapped source of fresh, infection free whole blood, if collected aseptically after the birth of healthy newborns from consenting mothers, and it has all the potentialities to be a ready replacement for blood loss .

In this connection it is worth mentioning another recent collaborative work of the University of Liverpool, U.K., and Komfo Anokye Teaching Hospital at Kumashi, Ghana, on the use of placental umbilical cord blood. They reported a substantial decrease in the mortality of children in sub-Saharan Africa suffering from severe anemia after falciparum infection, with the use of cord blood. (Ref 23, 24).

Conclusion:

In a report of the World Health Organization, it was revealed that there are about 500,000 pregnancy related deaths globally, of which at least 25 percent maternal deaths are due to the loss of blood. (Ref 25)
An estimated 13 million units of blood worldwide are not tested against human immunodeficiency viruses or hepatitis viruses, and in some developing countries 80 percent of the blood supply comes from paid donors or replacement donors (family friends or acquaintances) even when the infected population is high. (Ref 26)

For the last 70 years since the publication of the report of Amberson, (Ref 27) there have been global attempts to find a genuine blood substitute. Fetal hemoglobin is a natural stress response to hemoglobin synthesis which we try to preserve and augment in case of thalassemia by providing hydroxyurea or other similar drug supports. Other conditions like pregnancy, diabetes, thyroid disease, or anti-epileptic drug therapy, can also increase the fetal hemoglobin concentration. This fetal hemoglobin, with its abundant source, i.e., the placenta (in India alone, there are more than 20 million placentas produced as afterbirth every year), is actually a cause of environmental pollution in many parts of the developing world because it attracts natural scavengers and spreads infection, unless aseptically treated, or incinerated. The western or the developed world has been working on the use of a tiny microscopical fraction of cord blood, i.e., CD 34 stem cells only (.01 percent of the nucleated cells of the placental blood). My team of doctors has been successfully transfusing this blood as an alternative emergency source of blood transfusion in the background of anemia and emaciation of any aetiology, i.e., from surgery to medicine from HIV, thalassemia to leprosy or from advanced cancer to patients with a crippling polyarthritis, etc since 1999 (Ref 28-35). We have applied for a global patent on the use of cord blood in these areas. In fine to combat the emergency requirement of blood in natural or man made disaster management, i.e., civil or military due to current waging war against terrorism, this precious hypoimmune fetal cells (36-39) with altered metabolic profile is a gift of the nature, entrapped inside the placenta which could be readily available source of blood not only in the underresourced countries in the world but in case of the genuine need for blood substitute anywhere in the world at crisis.

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(10) Oski F.A, Naiman J.L vide reference 5.


(16) Guyton A.C, Hall J.E. vide reference 6


(33) Bhattacharya N et al, “Umbilical cord whole blood transfusion in HIV patients with anemia and emaciation” http://bmj.com/cgi/eletters/327/7414/562-a#59738, 17 May 2004

(34) Bhattacharya N et al, “Utilization of a genuine blood substitute: A suggestion to the Medical fraternity in Iraqi Hospital” http://bmj.com/cgi/eletters/326/7391/675#30850, 30 Mar 2003


A preliminary study report of 123 units of placental umbilical cord whole blood transfusion in HIV positive patients with anemia and emaciation
Introduction:

Anemia is a frequent complication of HIV infection, and its incidence is associated with progression of HIV disease particularly anemia that does not resolve, is associated with shorter survival of HIV-infected patients. The prevalence of anemia decreased in the HAART era, but transfusion was claimed to be positively associated with risk of death, suggesting limiting use of transfusions in nonemergency situations by some investigators. Alternative could be once-weekly epoetin alfa on quality of life which may improve along with the hemoglobin levels in anemic human immunodeficiency virus infected adult receiving antiretroviral therapy. But the real problem of erythropoietin is the cost involved with it which is difficult for the patients of Africa and the major part of the developing world. The second alternative for the treatment of severe anemia is blood transfusion because anemia may have a role in disease progression and survival. Recovery from anemia has been linked to improved survival outcomes. But blood transfusion has been claimed to be associated with accelerated disease progression and mortality in patients with HIV infection, and review of related literature suggests that the mechanism for negative transfusion-associated outcomes may be transfusion-related immunomodulation.

Apart from the problem of immunosuppression, blood transfusion is a problem in severe anemia as it may trigger cardiac overload and failure unless adequate care and precautions are taken. We have reported earlier that fetal hemoglobin rich placental umbilical cord whole blood which is readily available from the discarded placenta, if collected aseptically after the birth of a healthy newborn at or near term, could be used as genuine blood substitute (Ref).

Whether this cord blood could be an emergency and safe substitute for adult whole blood in case of anemia necessitating blood transfusion in HIV positive patients was the main theme behind the present work.

Case Presentations:

(1). Mamata Manina (Date of diagnosis 8.1.2000) reported with weakness and fever along with loss of weight. Past history of tuberculosis presented with intractable fever with progressive loss of weight. Husband was affected first and 3yrs back i.e., 2000. Worked as a goldsmith at Bombay. Husband was detected HIV positive in STM where the patient presented with pleural effusion. Mamata’s two children aged 11 and 3 yrs are tested negative for HIV. Treatment with antiretroviral drug started from 5th July 2002. (Lamivudine 150 mg twice daily + Staphodine 30 mg twice daily + Neveraphine 200 mg twice daily).

Case (2). Sumitra Majhi. (P1+0, L.C.B…7yrs). Detected at Bombay due to rapid loss of weight and fever—her husband, Mr Biswanath Majhi was a goldsmith at Bombay who died 3yrs back (2000) without receiving any specific treatment for his HIV. Sumitra had a past history of tuberculosis 6yrs back (Chest Xray was positive and sputum was negative. Pt took Anti Kochs treatment for 1yr. Pt also started having 3 drug regimen for antiretroviral drugs from 12th May 2002.

Case (3) Manorama Mandal. Her husband was a goldsmith who resided in Bombay and was tested (+) for HIV in Bombay in 1995 and he started having treatment from Bombay. Manorama’s presenting features were multiple abscesses with fever, extreme weakness with maculopapular eruptions at the periphery, which was also detected (+) for HIV in Bombay. STM Calcutta reconfirmed the HIV status in Calcutta. She had anti Koch’s treatment for one year after confirmation of the tubercular infection. In Calcutta antiretroviral drug support was initiated since 2002 (May 2nd)….died of gastroenteritis in July 2004.

Case (4) Palash Hazra……. Her presenting symptom was fever and extreme weakness in the background of diagnosed Pneumocystitis Carini from Bombay where he worked as a fabric designer in a nearby factory. He came to Calcutta and was detected Kochs (+) with investigations and was...
treated. His HIV status was detected (+) at STM Calcutta and reconfirmed in Medical college Hospital, Calcutta. He started receiving antiretroviral drugs since 29th April 2002 and drugs were discontinued thrice since its initiation. Pt has acute skin eruption with secondary infection all over the body, progressive loss of hair, dimness of vision, axillary and inguinal lymphadenopathy.

Case (5) Bharati Das.......husband was a goldsmith at Bombay......husband came from Bombay with fever, diarrhoea, weight loss, multiple ulcers 4 and half yrs back. Koch’s and HIV was detected (+) at Medical College and STM 4and half yrs back in 1999...treated with antikochs’ and ARV drugs for one and a half yr irregularly. Husband died 2yrs back in 2001. Pt was detected HIV(+) at STM in 2000 when she reported with progressive loss of weight and diarrhoea. She has two children aged 6yrs and 6yrs and are HIV(-). She started taking antikochs treatment for the last 10days after its detection however she could not afford to buy antiretroviral drugs as yet. She now complains of intermittent diarrhoea, hair loss, earache and toothache but there is no skin problem or weight loss.

Case (6) Soumen Routh is a jewellery worker at Bombay, returned to Calcutta with progressive weakness and fever and was detected (+) for HIV 1yr back......his brother also worked in Bombay as a goldsmith and was detected (+) for HIV in Bombay and died within 1yr of detection. Soumen did not have any feature of tuberculosis or history of antikoch’s treatment. His present problem is maculo papular rashes with intense itching all over the the body. He started having ARV since August 2003.

Case (7) Uma Das was detected (+) for HIV in 2001. Her husband died in Bombay due to HIV related cause in 2000. Uma reported to doctors in Bombay for her T uberculosis and after taking antikoch’s treatment she developed jaundice. Her only son Krishna (4yrs)old was HIV (+) since birth, she had a daughter (Puja) who was 8yrs old was also tested (+) for HIV had an episode of tuberculosis at the age of 1 and half yrs and was tr treated. Left for her alone Uma does not have residual means to buy ARV or AT drugs. Her present problem is intense skin reactions along with maculo papular rashes for the last six months.

Case (8) Sujata Das, husband, Sunil Das was confirmed to have HIV at Bombay eight yrs back. Her husband died of the disease 7 yrs back. She has a son aged 8 yrs who is not infected with HIV. She was diagnosed to be HIV (+) 8 yrs back at Bombay, she underwent partial treatment for HIV with ARV for 6 months back at Bombay. There was no past history of tuberculosis in either the mother or son. She developed maculo eruptive skin lesion which was treated conservatively.

Case (9) Dilip Bag, a goldsmith by profession worked in Bombay for 6 yrs. He presented with tuberculosis first and was treated 6 months for it in Calcutta. HIV was first detected in Calcutta and he is continuing treatment for HIV for last 6 months. He has no other problem at present.

Case (10) Ananda Mallick went to Bombay at the age of 12 and started working as a goldsmith. He used to visit the red light areas of Bombay occasionally. After a span of six yrs when he was of the age of 18 yrs he started having fever, headache, anorexia, etc. On facing these problems he came back to his hometown Calcutta. Here he was diagnosed to have been infected with Tuberculosis and HIV in the year 2001. Ananda took antikoch’s treatment for 1 yr but he was unable to procure ARV drugs because of poverty. He has skin disease only in his legs which has healed now. Ananda is unmarried.

Case (11) Manasha Ghosh is married and has a son aged 5yrs (who is HIV +ve). He used to work as a jewellery setter in Bombay from 1987 to April 2003. Manasha was tested HIV (+) at Bombay in April 2003 and came back to his residence immediately in Calcutta. He infected his wife in April 2003 and himself was found to be affected with tuberculosis also. Antikoch’s treatment was started in July 2003 and is continuing till date. No other member of his family has been infected with HIV. All other members of his family are aware that he is infected but in spite of this he is not socially neglected. ARV drugs has not yet been started.

Case (12) Sundari Ghosh was married in April 1986 and since then lived in Bombay with her husband Manasha. Both were diagnosed to be HIV (+) in April 2003 in Calcutta. She has not been affected with Koch’s. Sundari is not taking any ARV drugs.
Case (13) Tarun Ghosh went to Bombay in 1982 from there he went to Hyderabad in 2000 and stayed there for 2 yrs till 2002. After that he went to Vizag in 2003 and stayed there for 5 and half months. He suffered frequent attacks of diarrhoea in Vizag. He was confirmed to have HIV at Vizag. Tarun was married for 1 yr, his wife was not tested for HIV nor did she have any knowledge of her husband’s disease. He had antikoch’s treatment for last twenty days. He had mild respiratory problems and occasionally skin affections. He is having ARV drugs.

Case (14) Soma Pramanick was married in the yr 1993 to Pratap Pramanick. Her husband worked as a jewellery worker at Bombay from the age of ten till 1992. He suffered from lots of opportunistic infections and died in the year 1995. He was detected HIV (+) after marriage and Soma tested HIV (+) after childbirth but the child was tested HIV (-). She is getting antikoch’s and ARV drugs for the last two years.

Case (15) Biswajit Pal is married for eight yrs with no living issue. Working as an imitation jewellery worker for the last ten yrs in Domjur. He contacted a physician for psychological disturbance and loss of body wt. (he was 52 kgs initially but lost 10 kgs). Tested (+) for HIV 3 months back and is getting ARV drugs and antikoch’s.

Case (16) Tapas kumar Bose was suffering from rapid loss of weight and evening rise of temperature with progressive weakness. Came to Calcutta from his work place at Delhi and was detected to be positive for his HIV status. Patient is getting antikochs and antiretroviral drugs.
A HIV positive victim with anemia and emaciation is receiving cord blood transfusion. Two other HIV positive patients are waiting for their turn at the head end of the patient.
This is the flow analysis cytometry report of the peripheral blood CD34 level 72 hours after cord blood transfusion.

List of the HIV positive patients who received cord blood transfusion under the present series

<table>
<thead>
<tr>
<th>Sl. No. And Mode of transmission of HIV</th>
<th>Name, Age &amp; Sex</th>
<th>Blood Group</th>
<th>Primary presentation of the disease apart from anemia, i.e. Hb &lt; 8g percent or low</th>
<th>Transfusion of UCB: No of Units</th>
<th>Immediate reaction, viz, fever, chill and rigor, flank pain, back pain, blood in urine, fainting or dizziness</th>
<th>Late reactions like mild or progression to kidney failure, shock or delayed anemia</th>
<th>Complications like mild or moderate discomfort, anemia, shock, acute renal shutdown, lung dysfunction</th>
<th>Sense of well-being and weight gain</th>
<th>Unknown complication and rare complication like autoimmune disease or scleroderma due to microchimerism etc with followup till date</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Hetero</td>
<td>Age</td>
<td>Blood Group</td>
<td>Symptoms</td>
<td>Units</td>
<td>1st Transfusion</td>
<td>Last Transfusion</td>
<td>Notes</td>
<td></td>
</tr>
<tr>
<td>-----</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>M.M</td>
<td>23yrs</td>
<td>F</td>
<td>Fever, weight loss and diarrhoea</td>
<td>16</td>
<td>22.9.03</td>
<td>28.7.04</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>S.M.</td>
<td>7yrs</td>
<td>F</td>
<td>Fever and loss of weight</td>
<td>7</td>
<td>22.9.03</td>
<td>26.5.04</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>MM.</td>
<td>5yrs</td>
<td>H,F</td>
<td>Extreme weakness, fever and maculopapular eruptions</td>
<td>10</td>
<td>22.9.03</td>
<td>26.5.04</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>BD.</td>
<td>8yrs</td>
<td>H,F</td>
<td>Fever, weakness, loss of hair, Pneumocystis carinii Opportunistic infection</td>
<td>22</td>
<td>22.9.03</td>
<td>27.8.04</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>PH.</td>
<td>26yrs</td>
<td>M</td>
<td>Fever, diarrhoea, loss of weight and hair loss, generalized lymphadenopathy</td>
<td>9</td>
<td>28.1.04</td>
<td>16.4.04</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>SR.</td>
<td>40yrs</td>
<td>M</td>
<td>Weakness, fever, maculopapular rashes, itching</td>
<td>6</td>
<td>24.12.03</td>
<td>27.8.04</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>UD.</td>
<td>29yrs</td>
<td>F</td>
<td>Same as 6</td>
<td>3</td>
<td>13.12.03</td>
<td>12.5.04</td>
<td>Nil</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Hetero</td>
<td>Age</td>
<td>Blood Group</td>
<td>Symptoms</td>
<td>Units, 1st Transfusion</td>
<td>Last Transfusion</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>8</td>
<td>SD, 26yrs, F</td>
<td>O-</td>
<td>Rashes all over the body and weakness and fever.</td>
<td>17</td>
<td>13.12.03 and last on 29.8.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>DB, 20yrs, M</td>
<td>B+</td>
<td>Weight loss, fever</td>
<td>3</td>
<td>17.12.03 and last on 27.8.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>AM, 2yrs, M</td>
<td>O+</td>
<td>Maculopapular rashes and weight loss</td>
<td>3</td>
<td>24.10.03 and last on 24.12.03</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>MG, 3yrs, M</td>
<td>O-</td>
<td>Weakness and fever</td>
<td>2</td>
<td>28.1.04 and last on 27.8.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>SG, 27yrs, F</td>
<td>O+</td>
<td>Diarrhoea, loss of weight and skin rash</td>
<td>7</td>
<td>7.1.04 and last on 27.8.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>TC, 1yrs, M</td>
<td>A+</td>
<td>Loss of weight and fever and rashes</td>
<td>3</td>
<td>7.1.04 and last on 27.8.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>SP, 4yrs, F</td>
<td>AB+</td>
<td>Loss of weight, diarrhoea and fever</td>
<td>4</td>
<td>7.1.04 and last on 30.6.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>BP, 2yrs, M</td>
<td>O+</td>
<td>Loss of weight and fever</td>
<td>2</td>
<td>28.7.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>TB, 36yrs, M</td>
<td>B+</td>
<td>Loss of weight and fever</td>
<td>2</td>
<td>28.7.04</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Discussion: In the present study all sixteen cases had heterosexual transmission. The cases did not have any intravenous drug users or homosexual mode of transmission. 123 units of freshly collected placental Umbilical cord whole blood was transfused in 16 volunteers with HIV positive status with severe anemia and emaciation after getting necessary donor and recipients consent and passing through the institutional ethical committee. 75 percent of the cases had full blown AIDS. We did not encounter a single episode of any immunological and non immunological reactions during transfusion of the freshly collected blood from consenting mother undergoing caesarean section. 6 recipients were having B+ blood group followed by O+ in 5 patients and 3 cases were A+ and AB+ blood group was noted in 2 cases. The age of the group varied from 20yrs to 40years and 50 percent was male and the rest female. One patient received 22 units with 5units of blood at a time in a row, followed by 17 Units, then 16 units ,10 units, 2 cases received 9units each etc. Blood was transfused within 72 hours of collection to the victim on the basis of clinical priority availability and crossmatching, and after proper screening as per the standard blood transfusion protocol of WHO. Between the first blood transfusion to the last blood transfusion there is a gap of few months (4-10months) for periodic clinical evaluation from time to time. All the patients who received more than 3units of cord blood showed subjective and objective improvement in the form of less weakness, improved appetite and sense of wellbeing, even there is a gain in weight 3-5 pounds within the clinical observation period. In some case we did pre transfusion and 72 hour post transfusion CD34 study from the peripheral blood which showed a substantial rise. We are presently studying this effect of rise in CD34 in advanced immunodeficiency in HLA and sex randomized adults without getting the immunosuppressive drug support and its relationship with the CD4 and CD8 count.

In the animal kingdom swallowing the afterbirth by the mother is a general norm. Nature appears to have provided this precious wisdom to some of its creatures. Even herbivorous animals swallow the placenta after the birth of their babies (for example, the cow). But humans do not seem to know how to use this precious afterbirth, which has protected and nurtured the baby for so long in the womb. Of late, however, since 1989, (Ref 1, 2) global consciousness is increasing on the use of umbilical cord blood stem cells as an easily available source of hematopoietic stem cells for bone marrow transplantation. These fetal stem cells (CD 34) cause less graft vs. host reactions after transplantation. Recognition of this potentiality in the scientific world has resulted in the collection and harvesting of these cord blood stem cells in many laboratories all over the world. Nucleated cells of the But these hematopoietic stem cells constitute only .01 percent of the cord blood. The rest, that is, 99.99 percent of the cord blood is wasted. This wasted precious gift of Mother Nature is rich in fetal hemoglobin, growth factors and other cytokine filled plasma, and is moreover protected in the infection free environment inside the placenta in case of a healthy newborn.

Through evolution, the oxygen free carrying pigment in invertebrates became intracellular for better tissue perfusion in response to growth and metabolic demands from lower vertebrates to higher vertebrates. By incorporating the hemoglobin inside the RBC rather than carrying oxygen in plasma alone, the evolutionary process increased by about 100 times the oxygen carrying capacity of higher vertebrates. If hemoglobin is extracellular and intravascular, it may exert 5 times more osmotic pressure than plasma protein. As a result, water will be drawn from the tissue space and load the intravascular compartment. By inclusion of the hemoglobin inside the cell, the viscosity of the blood remains low, water is not drawn from the tissue space and the flow of blood with a large protein content is made possible. In addition, RBC membranes contain enzymes that protect the hemoglobin from degradation and allow it to work for three months at a stretch. A lack of enzymes in the membrane free hemoglobin leaves it at risk from oxidative damage by the haem molecule (Ref 3)
Furthermore, free hemoglobin is always subject to oxidative denaturation apart from acting as a trigger for hypertensive episodes.

In the global search for a suitable hemoglobin based oxygen carrier from human RBC to bovine RBC, or its chemically or genetically modified form, or even from sea creatures (Arenicola Merina), i.e., sea worm, the hemoglobin has been extracted (Ref 4) for its potential human use. Animal hemoglobin can trigger allergic reactions and can even damage the kidneys. Adult hemoglobin consists of 2 alpha and 2 beta polypeptide chains, each bound to a haeme group, capable of binding with one molecule of O2 (1 Gm hemoglobin binds with 1.39 ml of oxygen). Therefore, 14 gm percent of adult hemoglobin can carry, on an average, 19.46 ml of oxygen. Cord blood at term carries on an average 16.8 Gm percent hemoglobin (Ref 5), of which 20 percent belongs to the adult hemoglobin type (3.36 gms) and 80 percent belongs to the fetal hemoglobin type (13.44gms). The concentration of the fetal hemoglobin may increase further depending on fetal stress, maturity and several other feto-maternal factors. Fetal hemoglobin has the potentiality to carry upto 50 percent more oxygen than adult hemoglobin (Ref 6), i.e., 1Gm of fetal hemoglobin may carry upto 2.08 ml of oxygen. If we simply calculate theoretically the oxygen carrying potentiality of 100ml of cord blood taking into account of its 80 percent fetal hemoglobin component (2.08 ml O2 carrying capacity per gm of fetal hemoglobin) and 20 percent adult hemoglobin component (1.39ml O2 carrying capacity per gm of adult hemoglobin), it would be around 32.62 ml of O2 carrying capacity, which is a 67.62 percent additional oxygen capacity of the adult blood (19.46 ml Oxygen/100 ml). There are several factors which modify the O2 binding affinity, which includes, (a) concentration of hydrogen ion, (b) carbon dioxide concentration in the blood, (c) body temperature, (d) 2-3 diphosphoglycerate concentration only, to name a few.

In case of HIV infected patients, Allogeneic blood transfusions have been claimed to have an immunomodulatory effects and have been associated with activation of human immunodeficiency virus (HIV) and cytomegalovirus (CMV) in vitro and of HIV in small pilot studies. Retrospective studies suggest that transfusions adversely affect the clinical course of HIV. Data in selected non-HIV-infected patients requiring blood transfusion have suggested clinical benefit with leukocyte-reduced red blood cells (RBCs)(Ref). In general, as HIV disease progresses, the prevalence and severity of anemia increase. Anemia is also more prevalent in HIV-positive women, children, and injection-drug users than in HIV-negative women, children, and injection-drug users. Anemia has been shown to be a statistically significant predictor of progression to the acquired immunodeficiency syndrome and is independently associated with an increased risk of death in patients with HIV. Recently, the use of highly active antiretroviral therapy has also been associated with a significant increase in hemoglobin concentrations and a decrease in the prevalence of anemia. Treatment of anemia with epoetin-alpha has also resulted in significantly fewer patients requiring transfusion as well as decreases in the mean number of units of blood transfused but the real problem in the developing world in Asia and Africa is the cost involved in the procurement of safe HIV and hepatitis(B&C), malaria screened blood at nano level or molecular level by the PCR technology, or its alternative erythropoietin preparations. In case of healthy babies HIV cannot cross the placental barrier. The baby of the HIV positive mother is infected mostly by the extra placental mode of transmission of the infection(Ref).

Conclusion: In a report of the World Health Organization, it was revealed that there are about 500,000 pregnancy related deaths globally, of which at least 25 percent maternal deaths are due to the loss of blood. (Ref 25). An estimated 13 million units of blood worldwide are not tested against human immunodeficiency viruses or hepatitis viruses, and in some developing countries 80 percent of the blood supply comes from paid donors or replacement donors (family friends or acquaintances) even when the infected population is high. (Ref 26). India alone there are more than 20 million annual registered births take place and placenta is a biological waste everywhere. If we aseptically collect the blood from the placenta and use it in case of severe anemic victims of HIV with progressive emaciation, this HIV-related anemia has been shown to improve with cord blood transfusion in all the 16 cases of the present study. There is also definite improvement in the energy, and fatigue level in individuals with HIV, i.e., physical functioning, sense of well-being and weight gain from 2 pounds to 5 pounds within 3-10 months of the commencement of transfusion.
Reference:


A study on Placental umbilical cord whole blood transfusion—in 72 patients with anemia and emaciation in the background of Cancer

Introduction:

Anemia is the commonest hematological abnormality seen in cancer patients which increases with the progression of the disease. The frequency and the severity of the disease depends on the stage, grade, duration of the disease, current and the previous treatment protocol and the outcome of the therapy. Correction of anemia often improves the quality of life of the cancer patients (Ref 1). Treatment options include administration of different hematopoietic growth factors, red cell transfusion, different erythropoietin preparations and dietary enrichment and regulations. In a report published from USA it has been noted that 37 percent patients were anemic (Hemoglobin less than
12Gm percent) prior to chemotherapy and an additional 41 percent patient become anemic during chemotherapy. These cases should be treated with RBC transfusion or erythropoietin. Problems of erythropoietin use includes cost, inconvenience of frequent injections, limitation of efficacy, bone marrow refraction, and other indication restrictions (Ref 2). Another unresolved issue of erythropoietin use is the potentiality to trigger thromboembolic manifestations (Ref 3). Certain cancer chemotherapy is notorious for triggering anemia through its bone marrow suppression effect. Radiation and rapid tumor progression can also trigger anemia through marrow suppression and or infiltration or refraction. Under normal circumstances the antitumor effect of radiation is mediated through interaction with oxygen to form labile free radicals, intratumoral oxygen level has also a direct role on radiation induced tumor kill potential apart from the type of the tumor cell and its constituents’ composition. If not corrected before initiation of radiation anemia and subsequent tumor cell hypoxia can reduce the tumorcidal effect of radiation, in general apart from the negative effect of anemia on the quality of life of the cancer victims with progressive and worsening fatigue and depression (Ref 4). Patients with malignant diseases often require transfusion for relief of the symptoms of anemia. In most of the cases marrow function may be severely depressed by chemotherapy, radiotherapy. Infections and thrombocytopenic bleeding may be present and the recovery of the marrow is delayed due to combination of drug, malignant infiltration or poor nutrition. Physiological adjustment to chronic and acute anemia have a limit and particularly in elderly patients with myocardial and vascular disease anemia is poorly tolerated. The physician must decide when the patient is approaching this limit.

In our quest to solve the problem of anemia in patients of advanced cancer we looked for a solution everywhere. We noted that in the animal kingdom swallowing the afterbirth by the mother is a general norm. Nature appears to have provided this precious wisdom to some of its creatures. Even herbivorous animals swallow the placenta after the birth of their babies (for example, the cow). But humans do not seem to know how to use this precious afterbirth, which has protected and nurtured the baby for so long in the womb. Of late, however, since 1989, (Ref 1, 2) global consciousness is increasing on the use of umbilical cord blood stem cells as an easily available source of hematopoietic stem cells for bone marrow transplantation. Scientists seeking a suitable substitute of human blood tried for everything viz, from bovine RBC, or its chemically or genetically modified form, and others or even from sea creatures (Arenicola Merina), i.e., sea worm, the hemoglobin has been extracted (Ref 4) for its potential human use. Animal hemoglobin can trigger allergic reactions and can even damage the kidneys.

Human adult hemoglobin consists of 2 alpha and 2 beta polypeptide chains, each bound to a haeme group, capable of binding with one molecule of O2 (1 Gm hemoglobin binds with 1.39 ml of oxygen). Therefore, 14 gm percent of adult hemoglobin can carry, on an average, 19.46 ml of oxygen. Cord blood at term carries on an average 16.8 Gm percent hemoglobin (Ref 5) of which 20 percent belongs to the adult hemoglobin type (3.36 gms) and 80 percent belongs to the fetal hemoglobin type (13.44gms). The concentration of the fetal hemoglobin may increase further depending on fetal stress, maturity and several other fetomaternal factors. Fetal hemoglobin has the potentiality to carry up to 50 percent more oxygen than adult hemoglobin (Ref 6), i.e., 1Gm of fetal hemoglobin may carry upto 2.08 ml of oxygen. If we simply calculate theoretically the oxygen carrying potentiality of 100ml of cord blood taking into account of its 80 percent fetal hemoglobin component (2.08 ml O2 carrying capacity per gm of fetal hemoglobin) and 20 percent adult hemoglobin component (1.39ml O2 carrying capacity per gm of adult hemoglobin), it would be around 32.62 ml of O2 carrying capacity, which is a 67.62 percent additional oxygen capacity of the adult blood (19.46 ml Oxygen/100 ml). There are several factors which modify the O2 binding affinity, which includes, (a) concentration of hydrogen ion, (b) carbondioxide concentration in the blood, (c) body temperature, (d) 2-3 diphosphoglycerate concentration only, to name a few.

In India more than 20 million babies are born each year. We collected aseptically, fetal hemoglobin rich placental umbilical cord whole blood from the discarded placenta after the birth of the healthy baby, (which has the potentiality to carry more oxygen to the tissue Vol/Vol than adult blood because of its fetal hemoglobin component), at or near term, and used the same as an emergency substitute for adult whole blood after taking permission from the donor and recipient and passing through the Institutional Ethical Committee protocol. This project was submitted to the Dept. of Science and Technology, Govt. of West Bengal, Salt Lake, Calcutta in January 1999. The project was subsequently sanctioned vide order No 495/ST/P/S& T9G-1099 dated 25/3/1999.
Material and Methods:

Human placental umbilical cord blood was collected from consenting mothers aseptically after lower uterine Cesarean section under general or regional anesthesia. If there was gross prematurity or dysmaturity or the projected weight of the fetus was less than 2 kgs., or there was any specific disease of the mother like hepatitis or HIV, etc., the cord blood collection was abandoned. Cord blood was collected from only informed, healthy mothers with their consent after the birth of their healthy babies. The collection process started only after the baby was safely removed from the operation field and the anesthetist verified the stable physical condition of the mother. It was only then that the obstetrician took the decision to proceed with the umbilical cord blood collection. Immediately the cord was disinfected by spirit/Betadine solution at the site of the proposed puncture of the umbilical vein and a 16 g needle was attached to a standard pediatric collection bag (containing 14ml anticoagulant citrate phosphate dextrose adenine solution), which was used for the purpose of collection. A second bag was used if the collection exceeds or nears 100ml and a second prick was made at a proximal region after using a clamp at the first site of prick. The blood flows by gravity and generally within a minute 90 percent of the collection is over and within 2 minutes, in most of the cases, the blood flow ceases completely due to clot formation. In case of any confusion about the condition of the baby, the decision was immediately taken to preserve the blood in consultation with the paediatrician for future use by the baby, or stamped "Unsafe for transfusion", and no risk or chance whatsoever was taken for the eventual recipient of the blood.

When the collection was complete blood bag tubing was closed, sealed, and stored at 1-4 degree centigrade, after putting necessary identification markings. Another sample of the cord blood collected from the placenta was immediately tested for blood group (Rh and ABO), HIV(1 and 2), hepatitis B and C, VDRL, malaria as per standard blood transfusion protocol, which we reported earlier (7). Osmotic fragility study with .45 percent NaCl (N=40) at 4 ° centigrade, 35° and 40° with a time gap of 24 hours, 48 hours, 7 days and 14 days along with oxyhemoglobin (mmole/ml) and plasma hemoglobin (mg/ml) assessment in identical schedule showed in our laboratory that the cord blood was reasonably stable at room temperature. In case of any confusion/contamination, the culture was put aside for identification of the pathogen if any, through appropriate protocol, and the sample was stamped unfit for transfusion. In the present series, the collection of the blood varied from 54ml -128 ml mean 82 ml+7.6 ml SD , mean packed cell volume 48 + 4.1 SD, mean hemoglobin concentration 16.4 Gm percent + 1.6 Gm percent SD. After collection the blood was immediately preserved in the refrigerator and transfused within 72 hours of collection. Donation of the cord blood to the recipient followed the strict guidelines of the human ethical committee of the Hospital headed by an emeritus Professor of Medicine. As a rule, the volunteer who wishes to enroll for the cord blood transfusion programme, must have a hemoglobin count which is below 8 Gm percent. Before the umbilical cord blood transfusion, a thorough clinical examination of the recipient was done, including the proper monitoring of the BP/Pulse/Respiration and other cardinal and presenting features. Then pre- transfusion, a little blood was drawn from the prospective recipient of cord blood for Blood grouping, Hb/ Tc/ Dc/ ESR/ Platelet/ Coombs test, C-Reactive protein, Urea, Creatinine, Bilirubin and other investigations as per the requirements of the case. A little blood was redrawn from the same patient who received cord blood transfusion, after 24 hours, 72 hours, 7days, 1month, 2 months. 3 months and subsequently, clinical follow-up continued at the OPD from time to time, to study the effect of transfusion and adverse reactions if any. Actual transfusion procedure started after necessary grouping and cross-matching of the specimens and checking the identity of the patient. The cord blood was transfused by a blood transfusion set containing a filter (230 um). For the initial 15 minutes or so the patient was carefully observed to see if there was any transfusion reaction. Thereafter, if all went well, the transfusion rate was increased till it was completed.

Result and Analysis:

In the present series we are presenting the data of 213 units of aseptically collected cord blood from consenting mothers undergoing LUCS from 1st April 1999. Transfusion services and effective follow
up have been continued in the outpatient department to date. The blood was transfused to 73 informed consenting volunteers after the cases were passed through the institution based ethical committee.

The blood volume of a term fetus is approximately 80 - 85 ml/kg ([8]). The placental vessel at term contains approximately 150 ml of blood (Ref 9). The cord blood contains three types of hemoglobin, HbF, HbA, HbA2, of which HbF constitutes the major fraction (50-85 percent) (Ref 10). HbA accounts for 15 - 40 percent of hemoglobin and HbA2 is present only in trace amounts at birth (Ref 11). HbF has a greater oxygen affinity than HbA (Ref 12). The oxygen tension at which the hemoglobin of the cord blood is 50 percent saturated is 19-20 mm of Hg, 6-8 mm Hg lower than that of normal adult blood. This shift to the left of the hemoglobin oxygen dissolution curve results from poor binding of the 2,3-diphosphoglycerate by HbF (Ref 13, 14). The potential complications of blood transfusion therapy can be grossly divided under two headings, immunological and non-immunological reactions (Ref 15). The immunological reactions are related to the stimulation of antibody production by the foreign alloantigens by the different components of transfusion, e.g. RBC, leucocytes, platelets and plasma proteins. All immunizations may lead to immunological reactions in case of future stimulation by a similar antigen. The commonly encountered immunological reactions are haemolytic reactions due to red cell incompatibility. Febrile or pulmonary reactions are related to antigens of leucocytes and platelets. Allergic and anaphylactoid reactions are related to antibodies and it is only very rarely that we can see graft vs host reactions due to engraftment of the transfused lymphocytes in case of immunosuppression. The commonly encountered non-immunological reactions are because of physical or chemical properties of the transfused blood /blood products due to bacterial or viral contamination or the circulatory load.

During our experience of transfusion of 213 units of cord blood over the last five years, we have not encountered a single episode of immunological or non-immunological reaction so far. Fetal hemoglobin can carry more oxygen than the mothers blood and there is a potential advantage of the fetal hemoglobin (Bohr's effect) by which it can carry more oxygen at low PCO2 than at high PCO2 (Ref 16). Another potent advantage of cord blood transfusion which has therapeutic implication, is the rich cytokine and growth factor filled plasma in the cord blood, which eventually has a positive effect on distressed and emaciated patients. On the basis of our experiences, we can say that cord blood transfusion is safe and can be used in oncological patients at the time of need, as an alternative to adult whole blood transfusion, not as an inferior method of transfusion but as an effective supplementation of blood, which has no transfusion related hazards detected so far.

FIG-1

![Cancer Patients by Gender](image-url)
Fig 2

Number of Cases with Stage of Cancer

- STAGE 1: 10%
- STAGE 2: 19%
- STAGE 3 and 4: 71%

Fig 3

Distribution of Cases Showing correlation between Age and Number

<table>
<thead>
<tr>
<th>Age of the Patients</th>
<th>Number of Cases</th>
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Fig-4

Fig-5
Discussion: Continuous supply of donated blood is vital for the practice of modern medicine, but due to an ever increasing worry over blood borne diseases like HIV, hepatitis or bovine spongyform encephalitis in certain areas, has fuelled the search for an alternative source for blood transfusion. Hemoglobin based oxygen carriers have an intrinsic advantage of universal compatibility and storability at room temperature, because of the high cost involved, these would be simply unacceptable to the developing world in particular. Moreover, there are also specific problems of hypertensive impact, gastric irritability and unexplained deaths as reported in a trauma trial on the treatment of severe hemorrhagic shock. (Ref 18)

The other hemoglobin substitutes with lesser importance include perfluorocarbons, i.e., fluorine substituted with linear or cyclic carbon atoms with high oxygen carrying capacity, and liposome encapsulated hemoglobin (Ref 19).

Transfusion of adult blood is never a zero risk event anywhere in the world. Risks associated with adult blood transfusion include transmission of HIV (1 & 2), hepatitis B, C, A, G, Parovirus 19, which is especially of concern in case of pregnancy, hemolytic anemia and immunocompromised background, apart from the possibility of transfusion of syphilis, kalaazar, malaria (in the developing world), unless the blood is thoroughly screened as per WHO and country specific guidelines. There are also problems of rare blood groups which are not screened normally but have the potentiality to trigger hemolytic reactions. There are many other reasons of transfusion specific acute or delayed immunological and non immunological reactions, contamination problems with platelet, RBC, etc. Very rarely, there could be an incidence of transfusion induced lung, liver or kidney injury. Lastly, there could also be problems due to immunomodulation. (Ref 20). Newly identified, but well known, potential risk factors include the possibility of the transmission of Creutzfeldt Jakob disease in its classical or variant form, even after leucodepletion (lymphocytes are possible source of transmission of infection) as reported in an editorial article in BMJ (Ref 21).

Due to disease or treatment of cancer patients are often become immuno-compromised and thus become predisposed to wide variety of bacterial, viral and fungal infection and an allied cellular mediated immune response. Advanced cancer patients by virtue of their frequent exposure to transfusion develop HLA alloantibodies which can adversely affect the therapy, for example refractoriness of platelet transfusion. Thus cancer patients should ideally receive specially processed
blood products, for example, leucoreduced, irradiated cytomegalovirus seronegative blood products. Leucoreduction can prevent febrile non hematological reactions including HLA alloimmunization, antibody platelet reaction and subsequent refractoriness of the platelet transfusion, and also prevention of the cytomegalovirus transfusion only to name a few advantages. Blood components are irradiated to prevent the potentially lethal transfusion induced graft vs host disease by interfering with the ability of the lymphocytes to proliferate.

Standard guideline for goodstanding practice is the implementation of the recommendation the red blood cells, platelets and granulocytes with a minimum dosage of 2500 cCi radiation. (Ref: Guideline for the gamma irradiation of the blood components for the prevention of the transfusion associated graft vs host disease. BCSH blood transfusion task force. Transfusion Med. 1996;6:261.)

After our experience with 213 units of cord blood transfusion, we wish to affirm our faith in this safe transfusion protocol because we did not encounter a single case of immunological or non immunological reaction so far in any of our patients, even after the transfusion of 1 unit to 33 units (2838 ml on the basis of mean volume calculation) of cord blood to the same patient (with 10 units [mean 86x10 = 860 ml] of cord blood transfusion at a time) in different indications of blood transfusion) in the common background of anemia with malignant background disease. Our experience till date suggests that this placental cord blood transfusion could be an unique untapped source of fresh, infection free whole blood, if collected aseptically after the birth of healthy newborns from consenting mothers, and it has all the potentialities to be a ready replacement for blood loss.

In this connection it is worth mentioning another recent collaborative work of the University of Liverpool, U.K., and Komfo Anokye Teaching Hospital at Kumashi, Ghana, on the use of placental umbilical cord blood. They reported a substantial decrease in the mortality of children in sub-Saharan Africa suffering from severe anemia after falciparum infection, with the use of cord blood. (Ref 23, 24).

Conclusion:

In a report of the World Health Organization, it was revealed that there are about 500,000 pregnancy related deaths globally, of which at least 25 percent maternal deaths are due to the loss of blood. (Ref 25)

An estimated 13 million units of blood worldwide are not tested against human immunodeficiency viruses or hepatitis viruses, and in some developing countries 80 percent of the blood supply comes from paid donors or replacement donors (family friends or acquaintances) even when the infected population is high. (Ref 26)

For the last 70 years since the publication of the report of Amberson, (Ref 27) there have been global attempts to find a genuine blood substitute. Fetal hemoglobin is a natural stress response to hemoglobin synthesis which we try to preserve and augment in case of thalassemia by providing hydroxyurea or other similar drug supports. Other conditions like pregnancy, diabetes, thyroid disease, or anti-epileptic drug therapy, can also increase the fetal hemoglobin concentration. This fetal hemoglobin, with its abundant source, i.e., the placenta is actually a cause of environmental pollution in many parts of the developing world because it attracts natural scavengers and spreads infection, unless aseptically treated, or incinerated. The western or the developed world has been working on the use of a tiny microscopical fraction of cord blood, i.e., CD 34 stem cells only (0.01 percent of the nucleated cells of the placental blood). My team of doctors has been successfully transfixing this blood as an alternative emergency source of blood transfusion in the background of anemia and emaciation of any aetiology, i.e., from surgery to medicine from HIV, thalassemia to leprosy or from advanced cancer to patients with a crippling polyarthritis, etc since 1999 (Ref 28-35). We have applied for a global patent on the use of cord blood in these areas.

In line to combat the emergency requirement of blood for cancer patients, this precious hypoimmune fetal cells (36-39) with altered metabolic profile is a gift of the nature, entrapped inside the placenta which could be readily available source of blood not only in the underresourced countries in the world but in case of the genuine need for blood substitute anywhere in the world at crisis.
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Fig 4

Distribution of Cases Showing correlation between Age and Number

Number of Cases

<table>
<thead>
<tr>
<th>Age of the Patients</th>
<th>Number of Cases</th>
</tr>
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<td>1-10yrs</td>
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<td>71-80yrs</td>
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<td>81+</td>
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Fig 3

Number of Cases with Stage of Cancer

- STAGE 1: 10%
- STAGE 2: 19%
- STAGE 3 and 4: 71%

Fig-2
Spontaneous transient rise of CD34 cells in peripheral blood after 72 hours in patients suffering from advanced malignancy with anemia: Effect and prognostic implications of treatment with placental umbilical cord whole blood transfusion.
Introduction

Anemia is the commonest hematological abnormality seen in cancer patients. It increases with the progression of the disease (1). Correction of anemia often improves the quality of life of cancer patients (2). Corrective options include supplementation of different erythropoietin preparations and dietary enrichment and supplementation, and finally, red cell transfusion. Severe anemia can cause subsequent tumor cell hypoxia, which can reduce the tumorocidal effect of radiation in general (3,4,5,6,7). Advanced cancer patients, by virtue of their frequent exposure to transfusion, develop HLA alloantibodies, which can adversely affect the therapy, for example, refractoriness of platelet functions. Thus, cancer patients should ideally receive specially processed blood products, for example, leucoreduced, irradiated, cytomegalovirus seronegative blood products. Leucoreduction can prevent febrile non-hematological reactions including HLA alloimmunization. Blood components are irradiated to prevent potentially lethal transfusion induced graft vs host disease. Irradiation interferes with the ability of the lymphocytes to proliferate. A minimum dosage of 2500 cGy radiation is recommended for blood products before transfusion to a cancer patient to make the cells hypoantigenic and prevent alloantibodies and platelet refractoriness (8). Due to disease load or treatment, cancer patients are often immune-compromised and thus become predisposed to a wide variety of bacterial, viral and fungal infections and allied cellular mediated immune responses (9).

In our search for a solution to the problem of anemia in patients with advanced cancer, we examined viable readily available alternatives. We noted that in the animal kingdom, swallowing the afterbirth by the mother is a general norm. Even herbivorous animals swallow the placenta after the birth of their babies (for example, the cow). Nature appears to have provided a precious wisdom to some of its creatures. But humans seem to be unaware of the positive properties of the womb. There is up to 150 ml blood in the placenta, which has higher hemoglobin content than adult blood. It has a high fetal hemoglobin content, which is a normal stress response in pregnancy anemia, thyrotoxicosis, etc., and it can also carry more oxygen. If collected aseptically from healthy babies, after lower uterine caesarian section (LUCS), this blood can be used as an emergency source of fresh blood for transfusion purposes. This blood is hypoantigenic and the placental barrier is formidable. Even in cases of HIV infection, transmission occurs at the end of gestation through alternative routes, such as chorioamnionitis with leakage of the virus into the amniotic cavity or through trophoblast damage (10). Our work is based on the premise that placental umbilical cord blood can serve as a replacement for adult blood in cancer patients with anemia, and may have other multifaceted advantages. We have reported in this journal earlier about our experience with the transfusion of 413 units of freshly collected placental umbilical cord blood, in which we noted that not a single case of immunological or non immunological reaction was encountered (11).

In the present study, we have examined the fate of the CD34 in the placental blood which was transfused along with all other blood components to advanced cancer patients with anemia (Hemoglobin 8gm percent or less). This cord blood project was sponsored by the Dept. of Science and Technology, Govt. of West Bengal, Calcutta, from April 1999.

Material and Methods

Human placental umbilical cord blood was collected from consenting mothers aseptically after lower uterine caesarean section under general or regional anesthesia. If there was gross prematurity or dysmaturity or the projected weight of the fetus was less than 2 kgs, or if there was any specific disease that the mother was suffering from like hepatitis or HIV, etc., the cord blood collection was abandoned. Cord blood was collected from only informed, healthy mothers after the birth of their healthy babies. The methodological details of the cord blood transfusion protocol has been reported by us earlier (12). Flow analysis cytometry was done routinely for estimating the CD34 level of the peripheral blood three days after the transfusion of the cord blood in sex and HLA randomized patients from Ranbaxy Laboratories. No patient received any growth factor or specific immunosuppressive drug during the cord blood transfusion.

Result and Analysis
Figure 1: Serial CD34 flow analysis cytometry report of AP (A patient suffering from clinical stage 4 Non-Hodgkin’s lymphoma)

Figure 2: Serial CD34 flow analysis cytometry report of AC (A patient suffering from clinical stage 4 Carcinoma breast)
Figure 3: Serial CD34 flow analysis cytometry report of KC (A patient suffering from clinical stage 4 Carcinoma Lung)
Figure 4: Serial CD34 flow analysis cytometry report of BS (A patient suffering from clinical stage 4 Carcinoma breast)
Figure 5: Serial CD 34 flow analysis cytometry report of DS (A patient suffering from clinical stage 4 Carcinoma neck gland)

Figure 6: Serial CD 34 flow analysis cytometry report of UB (A patient suffering from clinical stage 4 Carcinoma Breast)
Figure 7: Comparison of Serial CD 34 flow analysis cytometry report of different patients suffering from advanced malignancy
Six cases of advanced malignancy (stage IV disease) were enrolled in the protocol of the study of the fate of hematopoietic stem cells (CD34) after placental umbilical cord whole blood transfusion (as assessed from the peripheral blood CD34 level, 72 hours after cord blood transfusion). All transfusion related CD34 of the peripheral blood were analyzed between 16th August 1999 and 16th May 2001.

The study group included three male and three female patients, suffering from clinical stage IV malignancy vide Table 1. Each one also had anemia (Hemoglobin <8gm percent), and received freshly collected placental umbilical cord blood to combat this. They also received standard treatment, i.e., surgery, radiation and chemotherapy, for their stage and grade of illness in addition of the transfusion protocol.

Case 4, was a patient suffering from Sarcoma breast, who received 6 units of cord blood, which is the lowest amount transfused in this group (Fig 4). Case 6, who had Cancer breast received the highest amount – 32 units (Fig 6). The youngest patient (Case 1), suffering from non Hodgkin lymphoma, was a 16 years old boy, who received 8 units of cord blood (Fig1). Case 5 (metachronous metastasis lymph node neck) received 15 units (Fig 5), Case2 (Cancer breast) received 14 units (Fig 2), and Case 3 (Cancer Lung) received 7 units (Fig 3). There were no transfusion related clinical, immunological or nonimmunological reactions.

Table 1: showing the list of patients suffering from advanced cancer who were followed up for their serial peripheral blood CD34 level after the cord blood transfusion

<table>
<thead>
<tr>
<th>AP</th>
<th>B+ve</th>
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Periodic assessment of the CD34 level from the peripheral blood revealed the trends noted below.

Case 1: In case of Non Hodgkin Lymphoma (Vide Fig. 1), the peripheral blood CD34 level showed an apparent rising trend up to 24 percent. The patient died within 3 months after the last transfusion, due to bronchopneumonia.

Case 2: In case of the patient with cancer breast (Vide Fig. 2), the peripheral blood CD34 level showed a declining trend from an initial rise after the first transfusion of cord blood. The patient died within 2 months after the last transfusion.

Case 3: In case of the patient with cancer lung (Vide Fig. 3), the peripheral blood CD34 level never crossed the base line. The patient died within 15 days after the last transfusion.

Case 4: In case of the patient with Sarcoma breast (Vide Fig. 4), after an initial hike, the CD34 level came down to the base level with slight marginal variation. The patient died within 7 months after the last transfusion.

Case 5: In case of the patient with metachronous metastatic bilateral neck nodes, there was marginal variation from the base line (Vide Fig. 5). The patient died within 21 days from the last transfusion of cord blood.

Case 6: In case of the patient with Ca Breast, there was a substantial rise after practically every unit of transfusion, reaching up to 99 percent (Vide Fig. 6) of the peripheral blood CD34 level (the normal
level of peripheral blood CD34 is less than 0.9 percent). This patient is living today without any clinical disease.

Fig. 7 compares the CD34 level in the six patients. The comparison and follow up at the OPD till date shows that the only patient who is living today without clinical disease is UB (Case 6). She received the highest number of cord blood transfusions, and it is in her that we noted a steep rise in the CD34 level after transfusion. In 4 other cases, there was very little variation or a downward trend in the CD34 level after an initial rise. In Case 1 (non-Hodgkin lymphoma), there was a slow increase, but the level never crossed 24 percent. On the other hand, in Case 6, it actually reached up to 99 percent.

There are many important factors which decide the fate of the malignancy and the host, i.e., from stage and grade of the disease, type of malignancy and the organ involved, age, background nutrition, modalities of treatment offered, and finally, the immune status of the host.

New approaches include immunotherapeutic strategies, but the type and extent of spontaneous immune responses against tumor antigens remains unclear. A dominance of TH2 cytokines in the patients’ sera, reported previously, suggests systemic tumor-induced immunosuppression, which potentially inhibits the induction of tumor-reactive T cells (13). Whether the freshly collected cord blood growth factor cytokine systems’ effect on the bone marrow, or the bone marrow rejuvenation by the CD34 rich cord blood transfusion, causes a transplantation effect due to background immune suppression in the advanced disease, is a matter under present study and follow-up.

Discussion
The persistence of donor leukocytes in the transfusion recipient is termed microchimerism (MC). It is likely that microchimerism reflects engraftment of the recipient with donor hematopoietic stem cells. This is very uncommon in transfusion for elective surgery, sickle cell anemia, thalassemia, and HIV (14,15). Long-term white blood cell (WBC) microchimerism of at least 2 years has been reported in trauma patients receiving fresh nonleukoreduced (non-LR) blood (16). A better understanding of factors determining clearance versus chimerism of transfused leukocytes is critical to the prevention of allogeneic responses and transfusion-induced graft-versus-host disease, and potentially, to the induction of tolerance for transplantation (17).

Pregnancy and neoplasm represent the most interesting examples of immune accommodation seen in mammalian biology. Cytokines of maternal origin act on placental development. At the same time, antigen expression on the placenta determines maternal cytokine patterns (18). In case of tumors, the expression of HLA-G protein on the surface of primitive melanoma and metastatic cells confers protection from natural killer (NK) cells and cytotoxic T lymphocyte (CTL) lytic activity (19,20). The placenta has a unique microenvironment and its sensitization impact on cord blood cells may have a role in transient transplantation impact on the host system. Tropheoblast cells of the placenta invade deep into the maternal uterine tissue to establish a life-giving connection with the maternal blood supply (21,22). The placenta is a complex organ that regulates maternal-fetal interactions (23). If we study the functional differences between an adult peripheral blood stem cell transplantation with a umbilical cord blood cell (UCBC) transplantation, the most important factor, apart from intrinsic differences, is the fact that hematopoietic stem cells (HSC) in UCBC have had a different set of microenvironmental exposures compared to those of adult marrow or the PBSC (peripheral blood stem cell) of adult blood. An example of differences between sources are some of the observed changes in HSC cell cycle status, gene expression and the adhesive and invasive properties induced by mobilization procedures used to generate PBSC, e.g., G-CSF (granulocyte colony stimulation factor).

This exposure of the hypoantigenic cord blood cells in the placental environment, (or this exposure to the hypoantigenic cord blood cells nurtured in the placental environment) along with the immune suppression (?) /immune mosaic state existing in the host system, either due to drugs, the chronic nature of the disease in advanced cancer, malnutrition with helminthiasis, reactivation of bacterial, viral or fungal diseases, or other associated causes like the impact of growth factors or selective cytokine impact of the cord blood on the bone marrow of the recipient, may help in the transient rise in the CD34 in the host. There was no clinical graft vs host disease in any of the cases. Our preliminary bone marrow study also suggested a positive impact on the host bone marrow with improved cellularity in those patients.
Conclusion

For continuation of the tolerance state, a certain degree of chimerism (coexistence of cells of genetically different individuals) is needed. This is best achieved if the inoculation contains cells capable of self-renewal, i.e., stem cells (25). In the present report, we have noted the results of freshly collected umbilical cord blood transfusion, and recognized that there is a transient rise of peripheral blood CD34 level (much higher than its normal level, i.e., up to .09 percent). The positive prognostic significance of this hitherto unreported unique phenomenon may be due to (a) non specific killing of the cancer cells by the CD34 cells of the donated cord blood, or (b) through induction of the dendritic cells (DC) of the cord blood, which are important accessory cells that are capable of initiating an immune response. The generation of functional DC from mononuclear cells isolated from human umbilical cord blood cells has already been reported. It has been shown that the cord blood-derived antigen-specific CTL can cause killing of human leukemic cells (K562) and breast cancer cells (MDA-231) (26). The other possibility (c) is the growth factor content or other specific cytokine components of freshly collected and transfused cord blood to the hosts’ bone marrow or the immune system.

Whatever might be the trigger, there is a transient rise in the CD34 cells of the peripheral blood up to 99 percent in one case in the bone marrow without provoking clinical graft vs host disease. This phenomenon has visible prognostic significance as can be seen particularly from Case no. 6 who is living today, i.e., 20th Nov, 2005. On the other hand, non-fluctuation of the CD34 after cord blood transfusion, resulted in early death (Case 3 and Case 5). The pathophysiology and clinical significance of this phenomenon is currently under our scientific scrutiny.

References:


PLACENTAL UMBILICAL CORD BLOOD TRANSFUSION IN TRANSFUSION DEPENDENT THALASSEMIA PATIENTS: A PRELIMINARY COMMUNICATION

Introduction

Thalassemia, also known as Cooley’s anemia, is an autosomal recessive genetic disorder affecting the hematopoietic system. It is characterised by anemia and a compromised hemoglobin transport throughout the body. It has various clinical ramifications depending on the stage and grade of the disease. For the most part, the disease is due to decreased production of the beta component of hemoglobin, i.e., the primary carrier of O2 in the blood. This disorder affects the people of the Mediterranean, the Middle East and South Asia for the most part. Beta thalassemia can be of three different types: Type A is mainly asymptomatic (trait) and is also known as thalassemia minor, which can present with mild anemia. Type B presents with hepatosplenomegaly and anemia along with growth failure features. Type C is the major variety of beta thalassemia which presents with severe anemia and complications of excessive iron load in the body, generally within one year of birth. In a general estimation, about 300,000 victims of thalassemia major can be detected globally. Treatment of beta thalassemia is essentially symptomatic with RBC transfusion to maintain a sufficient level of hemoglobin along with treatment of side effects of iron load by iron chelation therapy to remove
excess iron. This life long transfusion dependence can create many problems for the unfortunate thalassemic patient, though there are global attempts for making blood transfusions safer with stricter vigilance. There are also protocols for inactivation of microbes in platelet units, use of plasma with reduced viral activity and liberalization of the use of red cell substitutes (1). However, the risk of transmission of Creutzfeld Jakob disease in its classical or variant form even after leucodepletion, as lymphocytes are a possible source of transmission of infection (2). The problem is compounded in under resourced countries where blood transfusion itself can invite problems like HIV (1 & 2), hepatitis B, C, malaria, etc.

In the animal kingdom, swallowing the afterbirth by the mother is a general norm, thanks to Nature for providing this precious wisdom to its creatures. Even herbivorous animals swallow their own placenta after the birth of their babies, for example, the cow. But in the human system, we do not know how to use this precious afterbirth which protects and nurtures the baby in the womb for so long. Of late, since 1989, there is an increasing global consciousness on the use of umbilical cord blood stem cells as an easily available source of hematopoietic stem cells, for use in case of bone marrow transplantation, because it is easily available and it has a potential for inciting less graft vs host reaction due to its hypoantigenic nature. Many important laboratories in the world have been stimulated to collect cord blood for stem cell isolation and harvesting. But stem cells in the cord blood constitute .01 percent of the nucleated cells only, the rest, that is, .99 percent of the cord blood is discarded in the bin because it is seen as having no apparent use. This wasted precious gift of mother nature to the fetus has a high Hemoglobin, WBC and Platelet count and has growth factor and cytokine rich plasma which has been protected in the sterile environment of the womb under the constant showering of maternal blessings (antibodies) against all potentials maladies, i.e., infection. Whether this placental umbilical cord blood could be a readily available safe source of blood substitute against adult blood transfusion is the principal theme behind the present work, which received a grant from the Department of Science and Technology, Govt of West Bengal, Bikash Bhavan, Salt lake, Calcutta, India. The present paper deals with the problem of thalassemics with severe anemia who needed immediate blood transfusion support but could not arrange fresh concentrated RBC from any source. They applied to our institute based ethical committee for placental umbilical cord blood transfusion as an emergency procedure to combat the crisis.

Materials and Methods

92 units of human placental umbilical cord blood was collected from consenting mothers aseptically after lower uterine Caesarean section under general or regional anaesthesia. If there was gross prematurity or dysmaturity or the projected weight of the fetus was less than 2 kgs., or there was any specific disease of the mother like hepatitis or HIV, etc., the cord blood collection was abandoned. Cord blood was collected from only informed, healthy mothers with their consent. The collection process started only after the baby was safely removed from the operation field and the anaesthetist verified the stable physical condition of the mother. It was only then that the obstetrician took the decision to proceed with the umbilical cord blood collection. Immediately the cord was disinfected by spirit/Betadine solution at the site of the proposed puncture of the umbilical vein and a 16 g needle was attached to a standard pediatric collection bag (containing 14ml anticoagulant citrate phosphate dextrose adenine solution), which was used for the purpose of collection. A second bag was used if the collection exceeded or neared 100ml and a second prick was made at a proximal region after using a clamp at the first site of prick. The blood flowed by gravity and generally within a minute, 90 percent of the collection was over and within 2 minutes, in most of the cases, the blood flow ceased completely due to clot formation. In case of any confusion about the condition of the baby, the decision was immediately taken to preserve the blood in consultation with the paediatrician for future use by the baby, or stamped "Unsafe for transfusion", and no risk or chance whatsoever was taken for the eventual recipient of the blood.

When the collection was complete, the blood bag tubing was closed, sealed, and stored at 1-4 degree centigrade, after putting necessary identification markings. Another sample of the cord blood collected from the placenta was immediately tested for blood group (Rh and ABO), HIV (1 and 2), hepatitis B and C, VDRL, malaria, as per standard blood transfusion protocol, which we have reported on earlier (3).

In case of any confusion/contamination, the culture was put aside for identification of the pathogen if any, through appropriate protocol, and the sample was stamped unfit for transfusion. In the present series, the collection of the blood varied from 57ml -136 ml mean 84 ml+7.2 ml SD, median 87 ml, mean packed cell volume 45 + 3.1 SD, mean hemoglobin concentration 16.4 Gm percent + 1.6 Gm percent SD. After collection, the blood was immediately preserved in the refrigerator and transfused within 72 hours of collection. Donation of the cord blood
to the recipient followed the strict guidelines of the human ethical committee of the Hospital headed by an emeritus Professor of Medicine. As a rule, the volunteer who wishes to enroll for the cord blood transfusion programme, must have a hemoglobin count which is below 6 Gm percent. Before the umbilical cord blood transfusion, a thorough clinical examination of the recipient was done, including the proper monitoring of the BP/Pulse/Respiration and other cardinal and presenting features. Then pre-transfusion, a little blood was drawn from the prospective recipient of cord blood for Blood grouping, Hb/ To/ Dc/ ESR/ Platelet/ Coombs test, C-Reactive protein, Urea, Creatinine, Bilirubin and other investigations as per the requirements of the case. Hb electrophoresis was done before and after the transfusion to see the impact of transfusion. Actual transfusion procedure started after necessary grouping and cross-matching of the specimens and checking the identity of the patient. The cord blood was transfused by a blood transfusion set containing a filter (230 um). For the initial 15 minutes or so the patient was carefully observed to see if there was any transfusion related reaction. Thereafter, if all went well, the transfusion rate was increased till it was completed.

Result and Analysis

Adult hemoglobin consists of 2 alpha and 2 beta polypeptide chains, each bound to a haeme group, capable of binding with one molecule of O2. 1 Gm hemoglobin binds with 1.39 ml of oxygen. Therefore, 14 gm percent of adult hemoglobin can carry, on an average, 19.46 ml of oxygen. Cord blood at term carries on an average 16.8 Gm percent hemoglobin (4) of which 20 percent belongs to the fetal hemoglobin type (HbF) and 80 percent belongs to the fetal hemoglobin type (13.44 Gm percent). The concentration of the fetal hemoglobin may increase further depending on fetal stress, maturity and several other feto-maternal factors. Fetal hemoglobin has the potentiality to carry up to 50 percent more oxygen than adult hemoglobin (5), i.e., 1 Gm of fetal hemoglobin may carry up to 2.08 ml of oxygen. If we simply calculate theoretically, the oxygen carrying potentiality of 100ml of cord blood, taking into account its 80 percent fetal hemoglobin component (2.08 ml O2 carrying capacity per gm of fetal hemoglobin), and 20 percent adult hemoglobin component (1.39ml O2 carrying capacity per gm of adult hemoglobin), it would have around 32.62 ml of O2 carrying capacity, which is a 67.62 percent additional oxygen capacity of the adult blood (19.46 ml Oxygen/100 ml). There are several factors which modify the O2 binding affinity, which includes, (a) concentration of hydrogen ion, (b) carbon dioxide concentration in the blood, (c) body temperature, (d) 2-3 diphosphoglycerate concentration only, to name a few. The blood volume of a term fetus is approximately 80 - 85 ml/kg (6). The placental vessel at term contains approximately 150 ml of blood (7). The cord blood contains three types of hemoglobin, HbF, HbA, HbA2, of which HbF constitutes the major fraction (50-85 percent) (8). HbA accounts for 15 - 40 percent of hemoglobin and HbA2 is present only in trace amounts at birth (9). HbF has a greater oxygen affinity than HbA (10). The oxygen tension at which the hemoglobin of the cord blood is 50 percent saturated is 19-20 mm of Hg, 6-8 mm Hg lower than that of normal adult blood. This shift to the left of the hemoglobin oxygen dissolution curve results from poor binding of the 2-3 diphosphoglycerate by HbF (11, 12).

The potential complications of blood transfusion therapy can be grossly divided under two headings, immunological and non-immunological reactions (13). The immunological reactions are related to the stimulation of antibody production by the foreign alloantigens by the different components of transfusion, e.g. RBC, leucocytes, platelets and plasma proteins. Alloimmunizations may lead to immunological reactions in case of future stimulation by a similar antigen. The commonly encountered immunological reactions are haemolytic reactions due to red cell incompatibility. Febrile or pulmonary reactions are related to antigens of leucocytes and platelets. Allergic and anaphylactoid reactions are related to antibodies and it is only very rarely that we can see graft vs host reactions due to engraftment of the transfused lymphocytes in case of immunosuppression. The commonly encountered non-immunological reactions are because of physical or chemical properties of the transfused blood /blood products due to bacterial or viral contamination or the circulatory load.

In the present series, 14 patients volunteered for the cord blood transfusion protocol after getting necessary clearance from the hospital based ethical committee. The age of the patients varied from 6 months to 38 years with male :female sex ratio 1:1. One patient received 23 units of transfusion (receiving 6 units at a time ) because she started to have sudden menarche. Another patient received 16 units of cord blood (receiving 8 units at a time) due to bleeding P/R (Source: hemorrhoid which was treated with ligation and interruption). All other patients received 2-8 units of cord blood, receiving at least 2 units a time. They had universal complications of malnutrition, along with growth retardation (4 cases), impaired liver function (4 cases), hypofunction of the marrow (3 cases ) leaving aside osteodystrophy, Elisa Tb positivity for IgA and IgM (2 cases), mitral stenosis and incompetence, irregularity of the period and hypothyroid, were the other complications. The hemoglobin concentration of the patients in the present series varied from 3.5 Gm to 5.9 Gm percent Mean 4.36 Gm percent. All the patients tolerated the procedure and not a single episode of immunological or non immunological reaction was encountered.Yet another interesting finding was that there was a subjective definite
sense of well-being in the recipients, much more than after their previous episodes of transfusions with concentrated RBC from adult sources.
Fetal hemoglobin can carry more oxygen than the mother's blood and there is a potential advantage of the fetal hemoglobin (Bohr's effect) by which it can carry more oxygen at low PCO2 than at high PCO2 (14). Another potent advantage of cord blood transfusion which has therapeutic implication, is the rich cytokine and growth factor filled plasma in the cord blood, which eventually has a positive effect on distressed and emaciated patients. On the basis of our experiences, we can say that cord blood transfusion is safe and can be used in hours of crisis from the pediatric to the adult age groups in thalassemic patients, as an alternative to adult whole blood transfusion, not as an inferior method of transfusion but as an effective supplementation of blood, which has had no transfusion related hazards detected so far.

This graph represents the hemoglobin electrophoresis results of a 2 year old boy with thalassemia major who received a single unit of placental umbilical cord whole blood transfusion which resulted in a rise of fetal hemoglobin from 10.4 percent to 22.4 percent.
List of the patients with Beta thalassemia with hemoglobin below 6 gm percent who received emergency Umbilical Cord Blood (UCB) transfusion in the present series

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Name, Age &amp; Sex</th>
<th>Hemoglobin</th>
<th>Other diseases in the background apart from hepatosplenic nuleomegaly</th>
<th>Transfusion of UCB: No. of Units</th>
<th>Immediate reaction, viz, fever, chill and rigor, flank pain, back pain, blood in urine, fainting or dizziness</th>
<th>Late reactions like mild or progressive kidney failure, shock or delayed anemia</th>
<th>Complications like mild to moderate discomfort, anemia, shock, acute renal shutdown, lung dysfunction</th>
<th>Unexpected/Unusual Complication</th>
<th>Unknown complication and rare complications? Autoimmune disease or scleroderma due to microchimerism etc with followup till date</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>R.H., 2 yrs.</td>
<td>5.9 gm</td>
<td>Growth retardation</td>
<td>16</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>Name</td>
<td>Age</td>
<td>Gender</td>
<td>Weight</td>
<td>Condition</td>
<td>Reason</td>
<td>Duration</td>
<td>Treatment</td>
<td>Comments</td>
</tr>
<tr>
<td>---</td>
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<td>-----------</td>
<td>--------</td>
<td>----------</td>
<td>-----------</td>
<td>----------</td>
</tr>
<tr>
<td>2</td>
<td>S.B</td>
<td>6 yrs</td>
<td>M</td>
<td>3.8g</td>
<td>Impaired liver function</td>
<td>P/R</td>
<td>6</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>3</td>
<td>K.A</td>
<td>1 yrs</td>
<td>F</td>
<td>4 gms</td>
<td>She started regular period after transfusion</td>
<td></td>
<td>23</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>4</td>
<td>G.S</td>
<td>8 yrs</td>
<td>M</td>
<td>5.2g</td>
<td>Osteodystrophy, bowing of the legs, deformed chest wall and growth retardation</td>
<td></td>
<td>8</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>5</td>
<td>T.R</td>
<td>6months</td>
<td>M</td>
<td>4.1g</td>
<td>Impaired liver function</td>
<td></td>
<td>2</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>6</td>
<td>H.K</td>
<td>1 yr</td>
<td>M</td>
<td>5.2g</td>
<td>Impaired liver function</td>
<td></td>
<td>4</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>7</td>
<td>U.C</td>
<td>3 yrs</td>
<td>F</td>
<td>4.2g</td>
<td>Hypoplastic anemia &amp; mitral stenosis and incompetence</td>
<td></td>
<td>4</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>8</td>
<td>T.M</td>
<td>1 yr</td>
<td>M</td>
<td>3.9g</td>
<td>Hypoplasia of marrow</td>
<td>Elisa Tb+</td>
<td>6</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>9</td>
<td>PK</td>
<td>5yr</td>
<td>M</td>
<td>3.8g</td>
<td>Growth retardation</td>
<td></td>
<td>4</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>10</td>
<td>S.S</td>
<td>yrs F</td>
<td>M</td>
<td>3.5g</td>
<td>Growth retardation</td>
<td></td>
<td>5</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>11</td>
<td>R.B</td>
<td>4yrsF</td>
<td>M</td>
<td>4 gms</td>
<td>Hypoplasia of Bone Marrow</td>
<td>Elisa Tb+</td>
<td>3</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>12</td>
<td>C.S</td>
<td>24,F</td>
<td>M</td>
<td>5.6g</td>
<td>Marrow hypoplasia and amenorrhea</td>
<td></td>
<td>4</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>13</td>
<td>A.C</td>
<td>2yrs,M</td>
<td>M</td>
<td>3.5g</td>
<td>Impaired liver function</td>
<td></td>
<td>3</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>14</td>
<td>S.B</td>
<td>7 yrs</td>
<td>F</td>
<td>4.4g</td>
<td>Elisa Tb+ and Hypothyroidism</td>
<td></td>
<td>4</td>
<td>Nil</td>
<td>Nil</td>
</tr>
</tbody>
</table>
Discussion and Conclusion

An estimated 13 million units of blood worldwide are not tested against human immunodeficiency virus or hepatitis viruses. In some developing countries, 80 percent of the blood supply comes from the paid donors or replacement donors (relatives, friends or acquaintances), where the degree of infection is high (15). Out of 500,000 pregnancy related maternal deaths globally, 25 percent deaths take place due to poor replenishment and lack of timely replacement of blood loss, as per the report of the World Health Organization (16). Continuous supply of donated blood is vital for the practice of modern medicine, but because of an ever increasing worry over blood borne diseases like HIV, hepatitis or bovine spongiform encephalitis in certain areas, the search for an alternative source for blood transfusion is going on. For the last 70 years since the publication of the report of Amberson, (17) there have been global attempts to find a genuine blood substitute.

Fetal hemoglobin is a natural stress response to hemoglobin synthesis which we try to preserve and augment in case of thalassemia by providing hydroxyurea or other similar drug supports. Other conditions like pregnancy, diabetes, thyroid disease, or anti-epileptic drug therapy, can also increase the fetal hemoglobin concentration. This fetal hemoglobin, with its abundant source, i.e., the placenta (in India alone, there are more than 20 million placentas produced as afterbirth every year), is actually a cause of environmental pollution in many parts of the developing world because it attracts natural scavengers and spreads infection, unless aseptically treated, or incinerated.

The western or the developed world has been working on the use of a tiny microscopical fraction of cord blood, i.e., CD 34 stem cells only (.01 percent of the nucleated cells of the placental blood). My team of doctors has been successfully transfusing this blood as an alternative emergency source of blood transfusion in the background of anaemia and emaciation of any aetiology, i.e., from surgery to medicine from HIV, thalassemia to leprosy or from advanced cancer to patients with a crippling polyarthritis, etc., since 1999 (18-25). We have applied for a global patent on the use of cord blood in these areas.

Recently, clinical scientists from the University of Liverpool (26, 27) in a collaborative work with Komfo Anokye Teaching Hospital at Kumashi, Ghana, published a report on the safe and life-saving use of cord blood in pediatric anemia. They reported a substantial decrease in the mortality of children in sub-Saharan Africa suffering from severe anemia after falciparum infection, with the use of cord blood.

In fine, to combat the emergency requirement of blood in thalassemics, these precious hypoinnune fetal cells (28,29, 30, 31) with altered metabolic profile are a gift of nature, entrapped inside the placenta, which could be a readily available source of blood not only in the under-resourced countries of the world, but also for those in genuine need of adult blood or a safe substitute anywhere in the world. This is specially true for patients with thalassemia, who have a requirement for safe blood transfusions.

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PLACENTAL & PREGNANCY STEM CELLS

Anjali Mehta M.D., Curtis Cetrulo M.D., Phillip Stubblefield M.D., Kyle Cetrulo

Everyone Is or Should Be Interested in the Placenta

The placenta may prove to be a non-controversial source of hematopoietic and mesenchymal stem cells as well as endothelial progenitor cells. A “cocktail” of these three elements might be used in the future to treat hypoxic ischemic encephalopathy (H.I.E.) in the peripartum period for neuroregeneration, and a combination of these cells might also be used to treat one of the more than 80 diseases that have responded to stem cell transplantation. (Table 1). Furthermore, these cells have the potential to treat degenerative diseases such as heart disease, endocrine disorders such as
diabetes, and neurodegenerative diseases such as stroke, Alzheimer’s disease, Parkinson’s disease and spinal cord injuries. These cells may also be useful in the treatment of orthopedic problems.

**Hematopoietic Stem Cells**

Hematopoietic stem cells (HSCs), present in umbilical cord blood, have been used in over 6000 umbilical cord blood transplants since the initial report in the New England Journal of Medicine in 1989 showed a successful treatment of Fanconi’s anemia. 2000 umbilical cord blood transplants occurred last year.

**Mesenchymal Stem Cells**

Mesenchymal stem cells have been found in the Wharton's jelly of the umbilical cord (Matrix cells) as well as in the first and third trimester chorion, first trimester amnion, villous stroma (placental chorionic villi) and amniotic epithelial cells and surrounding fluid. Mesenchymal cells from Wharton's jelly and the other sources listed above have a broader plasticity than previously thought and can differentiate into neuronal cells, adipocytes, chondroblasts, osteoblasts and myocytes.

**Endothelial Progenitor Cells**

Endothelial progenitor cells have been isolated from the umbilical vein as well as from the umbilical cord blood. These cells, also called angioblasts are capable of neovascularization in response to hypoxia and ischemia. The fetal cells found in maternal circulation even years after the pregnancy has ended possibly contain the potential to protect the mother from hypoxia and ischemia.

**Plasticity**

Plasticity is the ability of stem cells to give rise to progeny of different cell types. Embryonic stem cell differentiation reduces the potential that stem cells might give rise to cells of other tissue types. Embryonic stem cells are totipotent, meaning that they are able to give rise to all other fetal or adult cell types. Post-embryonic stem cells are multi- or pluripotent—that is, they only give rise to many, but not all, cell types. Stem cells in adult tissues generally only possess the ability to give rise to a limited number of cell types, usually one or two.

Another alternative to inherent plasticity is cell fusion, where a stem cell fuses with a somatic cell and the nuclear material of two cells is combined in one. Cell fusion has made it possible for tissue stem cells to give rise to cells of other tissue types, such as epithelial cells, neurons, or endothelial cells. Therefore it appears that stem cells present in tissue of one type may give rise to cells of a different cell lineage by either inherent plasticity or cell fusion. A 1999 article in The New England Journal of Medicine called “Blood to Brain and Brain to Blood” demonstrates that when neural stem cells (NSCs) are placed in bone marrow environments they become hematopoietic stem cells and when HSCs are placed in the central nervous system they become neural stem cells. (Figure 1)

**Embryology of the Placenta/Amnion, Amniotic Fluid and the Umbilical Cord**

In mammals, the allantois forms the umbilical cord and the mesodermal components of the fetal placenta (Figure 2). The amnion and chorion are derived from the embryo. The chorion is derived from the trophoblast and the amnion from the epiblast as early as 8 days after fertilization (Figure 3). Gastrulation occurs during the third week of gestation, and the process establishes all three germ layers including the ectoderm, the mesoderm and endoderm. Gastrulation begins on the surface of the epiblast and is responsible for the differentiation and specification of cell fate.

At the end of the second month of gestation, the yolk sac is in the chorionic cavity between the amnion and chorion. By the end of the third month, the amnion and chorion have fused and the uterine cavity is obliterated (Figure 4). The amnion continues to enlarge at the expense of the chorion and forms a primitive umbilical cord. Amniotic fluid is produced by the cells lining the amniotic cavity (the amniotic epithelial cells-AE) and from maternal blood. The fluid increases from approximately 30ml at 10 weeks gestation to 450ml at 20 weeks gestate and reaches 800 to 1000ml at term (38-41 weeks). Excess amniotic fluid (1500-2000ml) is termed polyhydramnios (amniotic fluid index of 25 or more or a single pocket of > 8 centimeters on ultrasound). Oligohydramnios or decreased fluid is
when there is less than 400 ml of amniotic fluid (an amniotic fluid index of 5 or less). The volume of amniotic fluid is replaced every three hours, with the fetus swallowing amniotic fluid beginning as early as the fifth month and adding fetal urine daily. The amniotic fluid serves as a cushion for the fetus within the uterine cavity, allowing the fetus to move about freely. The amnion also covers the umbilical cord which contains two arteries and a vein surrounded by Wharton’s jelly, which is rich in proteoglycans and protects the umbilical cord blood vessels. The umbilical cord develops from the primitive allantois and from the mesoderm components of the fetal placenta in conjunction with hematopoietic cell development. Hematopoietic stem cells are first seen in the yolk sac, then in the aortic-gonadotropin-mesonephric region in the hindgut of the developing embryo (Figure 2-5). As the placenta is developing, HSCs migrate to the fetal liver through the umbilical cord vessels and then return through the umbilical cord vessels to seed the fetal bone marrow (Figure 5–6). Because of the simultaneous development of the HSCs with the development of the placenta, the umbilical cord, the amnion and fetal vessels, HSCs appear to retain some of the pluripotent properties of the epiblast. These primitive cells get trapped in Wharton’s jelly or the matrix of the umbilical cord during their journey through the primitive umbilical cord and are present within the Wharton’s jelly of the umbilical cord, umbilical cord blood, the umbilical vein, amniotic epithelial cells, various placental tissues and amniotic fluid. These cells, including the matrix cells of the Wharton’s jelly, the amniotic epithelial cells, the mesenchymal stem cells from various placental tissues and amniotic fluid all retain the pluripotent / multipotent properties of early stem cells. The abundance of HSCs in various fetal/placental areas is of great interest (Figure 7).

The Ontogeny Of Stem Cells

Totipotent human embryonic stem cells, the ancestors of all the somatic and germ cells of the entire organism, are found soon after the cleavage of the zygote. Stem cells retain this totipotency in the pre-implantation embryo (E.3.5) but shortly before implantation, at the blastocyst stage, stem cells become committed to certain types of tissues. Stem cells at the blastocyst stage are considered to be pluripotent, capable of differentiating into the three cell types. The trophoderm is committed to forming the trophoblast of the placenta. The primitive endoderm is committed to forming the parietal and visceral endoderm. In the placenta, the primitive endoderm forms all cells in the embryo proper and some internal extra-embryonic membranes such as the allantois, the amnion, and the yolk sac mesoderm. After implantation, (E5-6) these stem cells undergo further commitment in the embryo proper as the embryonic ectoderm, mesoderm, and endoderm form, producing the final cellular diversity consisting of approximately 260 distinguishable cell types and an estimated total cell number of one trillion cells in an adult human. If the cell retains the potential to form multiple differentiated cells then the cell is considered multipotent. (Appendix B p xxvii Fig 1 A & B with explanation. Handbook of Stem Cells Volume 1).

Primordial germ cells (PGCs) are present proximal to the epiblast and migrate to and colonize the genital ridges (E6.5). (Figure 1)The first “blood islands” or sites of hematopoiesis are the extra embryonic yolk sac followed by the intrayonic aortic-gonad-mesonephros (AGM) region. The AGM region generates the adult hematopoietic system, harbors migrating primordial germ cells (PGCs) and perhaps produces populations of mesenchymal stem cells, vascular progenitors, and perhaps hemangioblasts.

From the AGM region a common unrestricted precursor migrates to the fetal liver through the allantois. The onset of placental HSCs activity coincides with AGM and yolk sac and precedes fetal liver and circulating blood. During or shortly after this migration, (E10.5–E11) and before the liver is involved (E12.5–12.5), these multipotent progenitors are trapped in the Wharton’s jelly or matrix of the developing placenta and umbilical cord. It remains possible that the placenta forms a niche for HSCs maturation and expansion and for HSCs mobilization and intraembryonic colonization. The placenta HSCS-pool expands during mid-gestation with greater than a fifteen-fold increase of HSCs compared to the AGM and the yolk sac (Figure 7).

The multipotent progenitors that are trapped or imbedded in the Wharton’s jelly or matrix of the umbilical cord can be extracted at delivery and form a unique source of stem cells. These “Jelli Cells” (JC) have been shown to survive xenotransplantation and to respond to local signals to differentiate along a neural lineage. Because these JC “get stuck” in the Wharton’s jelly at approximately day E9.5 of embryonic life, they are probably immunologically different than the hematopoietic stem cell found in umbilical cord blood and a rich source of very primitive cells. The immunogenicity of a stem cell depends on the expression of the major histocompatibility complex (MHC) genes. Human embryonic
stem cells have been found to express only low levels of MCH – 1 proteins inhibitory effect on NK cells. That these cells seem not to be recognized by NK cells suggests that these cells may not be readily rejected by NK cells.\textsuperscript{xi, xii} That these cells seem not to be recognized by NK cells suggests that these cells may not be readily rejected by NK cells.\textsuperscript{xi, xii}

The human fetus develops immunological competence around 9 to 15 weeks of gestation. Fetal cell-mediated and humoral immunity begin to develop by 9 to 15 weeks (63-105 days).\textsuperscript{xiii, xiv} Cells destined to become B cells differentiate from hematopoietic precursors in the fetal liver by 8 weeks gestation and in the fetal bone marrow by 12 weeks. Mature plasma cells expressing IgM appear at 15 weeks gestation while those secreting IgG and IgA are present by 20 to 30 weeks gestation. The migration of a common unrestricted precursor to the fetal liver through the allantois and trapping in the matrix of the umbilical cord occurs well before the fetus develops immunological competence. These cells are relatively non-immunogenic and engraft without stimulating significant immune rejection, perhaps the reason why male fetal progenitor cells can survive in their mothers as long as 27 years postpartum.\textsuperscript{xv, xvi} When placed in the microenvironment of the brain, they differentiate along a neural lineage.\textsuperscript{xvii, xviii}

Hematopoietic Stem Cells

In 1974, Hal Broxmeyer first described hematopoietic stem cells (HSCs) in human umbilical cord blood. He suggested using these cells for transplantation in 1982 and was involved in the first transplant performed in 1988 in a patient with Fanconi’s anemia.\textsuperscript{xx, xxi} Fifteen years later that patient was reported as free of the manifestations of the disease. As reported in the New England Journal of Medicine in 2004, there now have been over 6000 transplants using umbilical cord blood as the source of the stem cells. The journal also reported and reinforced the concept of the role of umbilical cord blood stem cells for transplantation in adults.\textsuperscript{xii} The use of umbilical cord blood stem cells is now state-of-the-art and the standard of care compares favorably with the gold standard of stem cells derived from bone marrow.

Some of the problems with umbilical cord blood stem cells, however, are low cell dose and delayed engraftment. The nucleated cell dose is the most important determinant along with HLA typing to determine the success and speed of engraftment. A nucleated cell dose of less than 2.5 times 10 to the 7th shows poor success for engraftment. In one study, only 25 percent of samples collected met this critical level.\textsuperscript{xii} For this reason, novel approaches to the use of cord blood transplantation have been suggested, including using double unit transplants, expanding stem cells, and combining the stem cells with mesenchymal (MSCs) components. Wagner and Barker have successfully used double cord blood transplants.\textsuperscript{xii} The expansion of stem cells in vitro has not proven to be successful. The potent combination of MSCs and HSCs from umbilical cord blood could address these problems and make transplantation and engraftment more successful.

Another important determinant regarding successful engraftment relates to the way the cord blood itself is collected. There is mounting evidence that collecting and storing the whole blood rather than a fractionated component results in more successful engraftment because less of the important cells are lost.\textsuperscript{xxv}

Mesenchymal Stem Cells

In human bone marrow transplants, both the MSCs and the HSCs are collected and transplanted. In a fractionated or separated cord blood sample many cells are lost, often including the MSCs component. A combination of MSCs and HSCs could potentially lead to more successful transplants. The placenta and umbilical cord are potential sources for these MSCs cells.

MSCs have been isolated from the Wharton’s jelly of the umbilical cord (umbilical cord matrix cells, or UCM), from first and third trimester chorion; first trimester amnion, as well as from villous stroma (placental chorionic villi).\textsuperscript{xiv} Weiss has attempted to characterize the cells from Wharton’s jelly as well as show that they can differentiate into neuronal cells.\textsuperscript{xv} These matrix cells expressed markers common to MSCs including CD 166, CD 105, CD 90, CD 73, CD 49e, CD 44, CD 29, CD 13, as well as MHC class I, but they are negative for CD 14, CD 34, CD 45, and MHC Class II markers. Portman-Lang has shown similar cells isolated from first and third trimester chorion, first trimester amnion, and villous stroma (placental chorionic villi). Recently, Kathy Mitchell reported that the matrix cells from Wharton’s jelly also expressed Oct-4 and nanog.\textsuperscript{xvii}
Mesenchymal Stem Cells (MSC) have been isolated from the Wharton’s Jelly, also known as the human umbilical cord matrix (UCM). These cells proliferate in culture and express markers found in other stem cells. Specifically, UCM cells express the transcription factors Oct 4 and nanog which are important for maintaining the undifferentiated, pluripotent state of embryonic stem (ES) cells. Oct-4 and nanog have previously been reported to be restricted to pluripotent cells. These Oct-4 expressing cells are found in the perivascular region of the umbilical cord. They can differentiate into multiple cell types including neuronal, endothelial and epithelial cells and are therefore candidates for cell-based therapies. In contrast to ES cells, but like umbilical cord blood cells, UCM cells display more immune tolerance and have been shown not to form tumors when injected into immunocompromised mice. These cells are more easily accessible than bone marrow MSC, are more abundant than the MSC found in cord blood and lack the ethical considerations of ES cells.

Recent reports have described the identification of pluripotent or multipotent stem cells from human placental cord blood and amniotic fluid. The pluripotent stem cells have been identified in cord blood, whereas multipotent mesenchymal cells have been detected in various placental tissues. Mesenchymal stem cells have also been isolated from amniotic fluid.

In the latest issue of Stem Cells there is an exciting report about the isolation of stem cells from amniotic epithelial cells. Miki has shown that amniotic epithelial cells isolated from human term placenta express surface markers normally present on embryonic and germ cells. Amniotic epithelial cells also express the pluripotent stem cell specific transcription factors octamer binding proteins (Oct-4). These cells do not express telomerase, are non-tumorigenic and have the potential to differentiate into all three germ layers –

- Endoderm (liver, pancreas)
- Mesoderm (cardio myocyte)
- Ectoderm (neural cells)

The author’s conclusion is that “amnio derived from term placenta after birth may be useful as a non-controversial source of stem cells for cell transplantation and regenerative medicine.”

Pluripotent stem cells have also been isolated from amniotic fluid. This heterogeneous cell population expresses markers for all three germ layers. These cells also show a high cell renewal capacity with > 300 population doublings.

**Endothelial Progenitor Cells**

Human Umbilical Vein Endothelial Cells.

The human umbilical cord is one of the most important sources of endothelial cells. The availability of these cells has played a major role in the development of the field of vascular biology. Perfusion of the human umbilical vein with collagenase results in a pure preparation of the single layer of endothelial cells that line this vessel. Initial passages of these cells, which are grown in the presence of heparin and pituitary extract, maintain nearly all of the features of native endothelial cells including the expression of endothelial cell specific markers such as von Willebrand factor and CD31, expression of receptors for growth factors, cytokines and vasoactive ligands and specific signaling pathways for vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) Transforming growth factor β (TGFβ), tumor necrosis fact α (TNFα) and angiotensin II. Human Umbilical Vein Endothelial Cells (HUVECs) have provided a critical in vitro model for major breakthroughs in molecular medicine, including seminal insights into cellular and molecular events in the pathophysiology of atherosclerosis and plaque formation. They have also provided a mechanism for the control of ischemic tissue in embryogenesis.

Monolayers of HUVECs have been used for the study of the interaction of leukocytes and macrophages with the endothelial cell layer resulting in the discovery of adhesion molecules, chemokines and kinases that mediate the interaction of inflammatory cells with the endothelial surface and their migration into the media. Monolayers of HUVECs have been generated on deformable surfaces or in chambers which allow the study of the effects of shear stress and pulsatile flow on cell signaling in order to reproduce the effects of blood flow on endothelial cell function in vivo. These monolayers have been used to identify transcription factors such as KLF2 which regulate endothelial adhesion molecule E-selection in response to stress and proinflammatory cytokines such
as TNFα that mediate changes in cell adhesion and migration which play a role in the early changes of atherosclerosis.\textsuperscript{1,3,11}

Angiogenesis plays a role in the pathophysiology of atherosclerosis, rheumatoid arthritis, diabetic retinopathy, psoriasis, and tumor growth.\textsuperscript{6} Angiogenesis involves the replications, migration and remodeling of endothelial cells in the process of tube formation. HUVECs have offered an important \textit{in vitro} model for the study of all three of these processes. Culture of HUVECs on Matragel, an extract of endothelial basement membrane, results in the formation of honeycomb-like structures that simulate tube TGF\textbeta. Inhibitors of angiogenesis on the formation and organization of honeycombs by HUVECs have helped elucidate the mechanisms by which these factors regulate both this process.\textsuperscript{12}

An important example has been the demonstration that HMG-CoA reductase inhibitors, cholesterol lowering drugs referred to as statins, exert a dose dependent effect on honeycomb formation and signaling pathways suggesting that statins might decreased progression of atherosclerosis and stimulate the revascularization of ischemic tissues via an effect on angiogenesis.\textsuperscript{9,13}

HUVECs continue to play a major role as tools in the study of mechanism, pathogenesis and therapeutics in the vascular system.

Endothelial progenitor cells, also called angioblasts, have been isolated not only from umbilical cord blood but also from the umbilical vein.\textsuperscript{14,15} "Endothelial progenitor cells have been shown to functionally contribute to neoangiogenesis during wound healing, vascularization after myocardial ischemia and limb ischemia, endothelialization of vascular grafts, atherosclerosis, and retinal neovascularization."\textsuperscript{16}

"Administration of cord blood CD 34 positive, peripheral blood mononuclear cells (PB-MNCs) accelerated epithelial progenitor cell (EPC) functions and increased incorporation into newly formed vessels twofold."\textsuperscript{17,18}

\textbf{Fetal Cells in Maternal Circulation}

Fetal cells can be detected in maternal circulation as early as six weeks of gestation, and by 37 weeks gestation, one-hundred percent of women have fetal cells circulating in maternal blood.\textsuperscript{19} The discovery of fetal cells that persist in maternal blood decades after delivery may provide a new paradigm that facilitates understanding the many possibilities of fetal cells.\textsuperscript{9,11} A new field is emerging studying fetal cell microchimerism, where fetal cells persist in maternal circulation and tissues. Findings suggest that the fetal cells may contain stem cell properties that are transferred to maternal blood. These cells, termed pregnancy associated progenitor cells, persist after delivery and may aid in a response to injury in the maternal system.

One case study of a woman with hepatitis C showed evidence that was convincing that fetal male cells were aiding the maternal system to control her disease. The patient stopped treatment with interferon and ribavirin against medical advice. DNA analysis suggested that the male cells were derived from a pregnancy with a male fetus that was terminated more than fifteen years earlier. The male cells from the liver biopsy were similar in morphology to surrounding liver cells, therefore making it likely that the male cells were derived from hepatocytes.\textsuperscript{20,21}

The discovery of fetal cells in the blood of women who have been pregnant suggests that fetal stem cells have a protective effect in women, even years after pregnancy. Diana Bianchi in a recent JAMA publication reported on this remarkable finding.\textsuperscript{22} The study examined tissue of ten women ranging in age from 34 to 37 years who suffered from a variety of diseases and who had had male offspring. Biopsies were taken from the thyroid, cervix, gallbladder, intestine, liver, spleen, and lymph node. The tissue samples were evaluated for morphology, cell surface, and intracellular phenotype of fetal cells in maternal organs. 701 male XY + microchimeric cells were identified in the tissue sections from the 10 women. 14-60% of the XY + cells in epithelial tissues (thyroid, cervix, gallbladder, and intestine) expressed cytokeratin, a marker of epithelial differentiation. In the epithelial cell samples, CD45, a common leukocyte antigen was expressed in the microchimeric cells associated with areas of inflammation or with the healthy tissue surrounding diseased tissue. In hematopoetic tissues, such as lymph nodes and spleen, CD45 was found in 90% of the XY + microchimeric cells. In the sample of liver tissue, one woman showed 4% of the XY + cells were consistent with hepatocytes. This study suggests that fetal cells found in women years after pregnancy not only persist, but may have multi-lineage capacity with the potential for a variety of protective functions.
By one estimate, if the umbilical cord blood and/or matrix cells were saved at birth, the probability of usage would be approximately 1 in 415 if degenerative diseases were included in the list of diseases that might be treated.\textsuperscript{lxv}

The treatment of chronic disease with stem cells has been referred to as a new field in medicine called Regenerative Medicine. The National Academy Committee report called “Stem Cells and the Future of Regenerative Medicine” provides a thorough review of the subject.\textsuperscript{lxvi}

Three of the most prominent areas of future treatment with stem cells include addressing neurological diseases, heart disease, and diabetes. A summary of the extensive literature on these three subjects follows below.

Endogenous neural stem cells (NSCs) have a poor regenerative ability. Neural stem cells therefore are ideally suited for the molecular and cellular therapy required by extensive, diffuse, and even global degeneration processes. Neurodegenerative conditions including myelin disorders, storage diseases, motor neuron degeneration, dementing conditions such as Alzheimer’s disease, and ischemic and traumatic pathology such as stroke would all be amenable to such treatment. Parkinson’s disease, Huntington’s disease and spinal cord injuries as well as cerebellar degeneration to the hindbrain appear to be more restricted in their involvement. Even these disorders, however, would be amenable to multiple neuronal cell type replacement by a migratory, responsive, multipotent neural progenitor because they require cell replacement.\textsuperscript{lxvii, lxxvii}

It is clear that cardiomyocytes can be transplanted into normal or injured adult hearts.\textsuperscript{lxxii} Cardiomyocyte transplantation is a paradigm for treating diseased hearts and has emerged as a potential therapeutic intervention, enhancing angiogenesis, providing structural support, and restoring lost myocardial mass in damaged or infarcted hearts. The sources of these cardiomyocytes have included angioblasts and vascular precursors, bone marrow and mesenchymal stem cells, skeletal myoblasts, and fetal cell-derived cardiomyocytes.\textsuperscript{lxix} Fetal cardiomyocytes have been demonstrated to directly participate in the functional synctium, and the transplantation is not associated with any anomalies in intracellular calcium handling.\textsuperscript{lxv}

The introduction of the Edmonton protocol in the year 2000 has provided encouraging results regarding the transplantation of pancreatic islet cells for the treatment of diabetes.\textsuperscript{lxxix} The success of this approach, which has now been used in over 100 patients worldwide, has allowed for much better glycemic control.\textsuperscript{lxxx} The search continues to find a new source of insulin-producing cells that can be used for transplantation.

Although there is much debate about the mechanism of plasticity, there is no debate that adult stem cells offer enormous potential to treat disease across all systems the human body.\textsuperscript{lx,i} There have been many studies with promising results focused on the nervous system.\textsuperscript{lxxii} Cells from cord blood and bone marrow have shown the ability to repair the liver and the heart.\textsuperscript{liii} These cells may become standard of care in many cancer therapy treatments. The phenomenon of cells adopting the behaviors of another system may take place secondary to cell fusion, plasticity or an undiscovered term. Regardless of how we define this process, these cells are changing medicine and the way that we will approach disease treatments in the future.

"Through a careful and circumspect series of experiments and trials we may learn whether we, indeed, have found within nature’s own tool box, a powerful and versatile therapeutic tool.”

- Rodolfo Gonzalez, P698, Handbook of stem cells vol. 1

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Umbilical Cord Blood Transfusion—a Clinical Overview

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Introduction

Fetal blood from the umbilical cord and placenta is a commodity which is wasted and disposed of after birth. Placental vessels at term contain an average of 150ml of blood (Haselhorst et al., 1930).

Children who develop cancers such as leukaemia and lymphoma often need bone marrow transplants following attempts to eradicate the cancer with strong chemotherapy and radiotherapy.

If matching bone marrow cannot be obtained from a relative, then the search for a match can be lengthy and often fruitless.

But stem cell from the subject’s cord blood which had been collected and stored will always be a perfect match, and can be thawed out and delivered in an autologous blood transfusion.

Recently there has been considerable interest in the use of cord blood as an alternative stem cell source to treat cancer and genetic diseases. More than 5000 cord blood transplantations of matched and partly matched blood, have been reported in the world literature.

Practical techniques have developed for collection, scrutiny and storage of cord blood. Indications for cord blood transfusion/transplantation are also expanding.

Characteristics of Umbilical Cord Blood

The yield of the cord blood varies from vol. 57 -134 ml mean 88 + 14 ml SD and mean haemoglobin 17.6 gm per cent (Bhattacharyya et al 2002b). This blood has a much higher haemoglobin (mostly fetal Haemoglobin), platelet and leukocyte content than adult whole blood. Additionally, it has a high concentration of Cytokine/Growth factors in its plasma, which eventually helps in the gene-switching mechanism after the birth of the baby.

This blood has a much higher oxygen carrying capacity than that of adult whole blood, and hence, the transfusion of fetal haemoglobin rich cord blood has the potential for better tissue perfusion of oxygen (vol/vol) to the recipient’s tissue than an identical volume of adult whole blood.
Compared with bone marrow cells, CD34+/CD3- cord blood cells proliferate more rapidly and generate larger numbers of progeny cells. Cultures of cord blood CD34+ cells increase in cell number every 7 to 10 days, several hundred fold greater than the increase in cultures of similar cells from adult bone marrow.

Cord blood contains a high proportion of T cells expressing the CD45RA+ /CD45RO-, CD62L+ "naive" phenotype (Szabolcs et al, 2003)

Some studies show that cord blood cells produce increased amounts of the anti-inflammatory Cytokine and Interleukin-10, which may down modulate graft versus-host disease (Bacchetta et al, 1994)

Indications for Cord Blood transfusion

There are two basic reasons for collection and transfusion of cord blood –

a. as a source of haematopoietic progenitors for allogenic stem cell transplantation in cases of leukaemia or bone marrow aplasia. The treatment has been undertaken in children as well as in adults.

b. as a source of haemoglobin in cases of anaemia for transfusion in Sickle cell Disease, HIV, Thalassaemia It has been suggested as a source for possible peri-operative blood transfusion.

It is possible to transfuse umbilical cord whole blood to older men and women after removing the stem cell content (.01 percent of the nucleated cells of the cord blood), to combat anaemia and raise immunity (Bhattacharya 2002b)

Umbilical Cord Blood Transfusion for Anaemia

Clinical indications which are expanding currently include cases of severe anaemia, renal or hepatic dysfunction and other conditions of diminished cardio-respiratory reserve or tissue hypoxic conditions, in any age group.

Hassall and colleagues (2003) lowered mortality of children with severe anaemia in sub-Saharan Africa by transfusing Umbilical-cord blood, with a mean volume of 85 mL (SD 28.0). This amount of blood is sufficient to raise the haemoglobin concentrations in 28 (21%) of 131 children requiring transfusions in the same hospital, by 30 g/L.

Bhattacharya and colleagues (2002b) reported on the use of Cord blood for transfusion in adults. They have transfused more than 350 units of freshly collected umbilical cord whole blood in different indications of adult blood transfusion, without encountering any immunological or non-immunological reactions.

Umbilical cord blood transfusion has also been suggested in cases of Thalassaemia, HIV infection with anaemia, and Leprosy with anaemia (Bhattacharya 2004)

Elderly patients

Umbilical cord blood transfusion had been carried out in elderly patients after removing the stem cell content (.01 cent of the nucleated cells of the cord blood), to combat anaemia. This cord blood is rich in fetal haemoglobin (which carries 60 percent more oxygen than adult haemoglobin), growth factors and cytokines, etc. and therefore has a potential growth promoting role. In the light of recent developments in molecular biology, it has been suggested that fetal stem cells and germ line cells, which express telomerase reverse transcriptase, can divide indefinitely and thus have the potential to increase cell numbers in a failing organ in the elderly after fetal cell transplantation and its homing effect on the hosts' organ (Bhattacharya 2002a).

Umbilical Cord Blood Stem Cell Transplantation
The indications are expanding. These can be considered under three headings:

A. Children with malignant disease. —Umbilical cord blood been used successfully in related transplants or with minor mismatch (with 1 or 2 loci unmatched) for both malignant and nonmalignant diseases. There is a low but definite incidence of Graft Vs. Host Disease (10 to 15 per cent). Cord blood, with low content of T cells is suitable for non-malignant diseases as there is no requirement for Graft vs Leukaemia effect. Engraftment occurs in over 80 per cent of cases. The event free survival at 24 months is about 30 to 40 per cent. (Balen, 2005)

B. Metabolic Disorders —Cord blood transplantation has also been shown to be effective in metabolic Storage diseases. There were 20 children with Hurler syndrome who received multiple chemotherapy followed by infusion of unrelated 1-, 2-, or 3-antigen-mismatched cord blood. With a median follow-up of 905 days, 17 of 20 children are alive with complete donor chimerism and normal peripheral blood alpha-1-iduronidase activity (Staba et al, 2004)

Cord blood transplantation can be successful, even if the patient and cord blood donor are mismatched at 2 antigen sites. Graft-versus-Host Disease is uncommon without a significant diminution of Graft vs Leukaemia response.

C. Adult cord blood transplantation -mismatched(unrelated) transplantation has been carried out in adults with leukaemia, lymphoma, and myelo dysplasia along with a variety of conditioning and GVHD prophylaxis regimens. The results indicate high transplant related early mortality mostly related to infection (47% death within 100 days), and 26 per cent disease free at 22 months follow up.

A comparison with unrelated but HLA matched bone marrow transplants, shows higher mortality but lower Graft-versus-Host Disease in the cord blood group. Survival of around 20 per cent was noted in recipients of unrelated cord blood and mismatched bone marrow. Indeed, studies suggest that outcomes are improved for patients receiving a matched unrelated bone marrow transplant.

Recipients of mismatched cord blood and 1-antigen-mismatched unrelated bone marrow had similar lower survival rates (Laughlin et al 2004)

Improving Results of Cord Blood Transplantation in Adults

Mortality and disease free survival in adult cord blood transfusion case can be improved by

a. pooled or sequential blood transfusion of a second partially matched cord blood unit
b. cord blood expansion using mixtures of Cytokines such as Stem Cell Factor, G-CSF, and Megakaryocyte growth factor etc.
c. combined cord blood and haploid identical CD34+ bone marrow transplants.
d. non-myeloablative or reduced strength conditioning regimens with one of the above.

Collection and Storage of Umbilical Cord Blood in health

Blood from the maternal donor is tested for infectious disease markers, including tests for syphilis, human T-cell lymphotropic virus 1 (HTLV-1), HIV, Hepatitis B, Hepatitis C, and CMV.
The blood is taken from the placenta and umbilical cord just after birth and then frozen under liquid nitrogen at -180°C. This blood contains "stem cells", which can be harvested and kept in a frozen state.

The cord blood can be collected either in utero, before the delivery of the placenta, or ex utero, after placental delivery. In utero collections are usually performed by the obstetrician or nurse midwife attending the delivery, while trained personnel from the cord blood bank, who perform the collection outside the delivery room, more often perform ex utero collections.

Potential Hazards

Recent research suggests that pre-cancerous cells can be found from birth onwards in children who go on to develop leukaemia.

If this is the case, returning umbilical blood to the same child may lead to renewed cancer a few years later and by that time, the child may be at an age where chemotherapy is not so effective.

Of course, if a match can be found with another baby's umbilical cord blood, no such difficulties arise. Otherwise, a matched bone marrow transplant is preferable to autologous cord blood transplant.

Other risks are infection and transmission of damaged cells (please see below).

Blood Banking Issues

There are issues related to recruitment and screening of donors, consent of the mother, testing and processing after blood is collected, freezing and storage and distribution to recipients. For a review please see Balen (2005).

Future Trends

There is a trend towards utilising cord blood instead of a matched unrelated donor, for example, in elderly patients with a high risk of graft-versus-host disease. There appears to be a less risk of a graft-versus-host disease. In this procedure additional application in non-malignant diseases is likely to be introduced in the future.

Another intriguing application is in HIV Disease worldwide public health problem, in which the possibility of gene transfer to a haematopoietic stem cell reservoir may eventually be possible. An allergenic stem cell vaccine may replace haematopoietic stem cells infected with HIV with uninfected umbilical cord blood cells (Goodwin et al 2001).

Another area of application could be autoimmune diseases, where there has been some success with autologous transplantation. The low risk of graft-versus-host disease makes cord blood transplantation of positive benefit in comparison with bone marrow transplant.
Further future areas of cord blood transfusion may be other non haematopoietic applications, such as the repair of damaged myocardium or neural tissue. Cord blood cells are a more primitive population than adult bone marrow, and have increased capacity for pluripotent differentiation.

However, caution is needed in this line of research. There are unknown potential risks of transmission of malignant, autoimmune and infectious agents (prions) through blood transfusion. In many cases screening tests do not exist and prevention of the passage of the harmful agent may not be possible (Braude et al., 2005). Treatment should be carried out only in recognized centres with quality control.

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Hematopoietic Stem Cells from Umbilical Cord Blood

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Hematopoietic Stem Cells

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Abstract

The use of umbilical cord blood stem cells is an efficient alternative for the transplantation of hematopoietic progenitor cells. Parameters commonly used to evaluate an umbilical cord blood unit and predict transplant outcomes have been total nucleated cells and CD34+ cells counts. Lack of CD38, HLA-DR and lineage committed antigens, as well as the co-expression of Thy-1 (CDw90), c-kit receptor (CD117), among others surface markers, have been shown to identify the hematopoietic stem cells in umbilical cord blood. A number of factors can influence the volume and amount of CD34+ cells, which are considered as immature and capable of proliferation. Quantification of CD34+ as well as correlations of such factors as maternal age, gestational age, newborn sex and weight, umbilical cord length, placental weight with increased volume and concentration of immature cells, among others are important issues that can influence the success of umbilical cord blood cells transplantation. Ex vivo culture is a crucial component of several clinical applications currently in development including gene therapy, and stem/progenitor cell expansion. By a way cord blood cells seem to be more interesting than bone marrow cells for being more immature, and thus "better stem cells", for presenting a lower frequency of graft versus host disease after transplantation, and for apparently being more susceptible to gene transfer. On the other hand, cord blood hematopoietic stem cells are enough to reconstitute children, however, sometimes, not to engraft an adult, requiring ex vivo manipulations.

Keywords

Umbilical cord blood, Hematopoietic stem cells, Surface molecules, Culture
1. Introduction

Over the last years, umbilical cord blood has been clinically investigated as an alternative source of hematopoietic tissue for allogeneic transplantation of patients lacking a human leukocyte antigen-matched marrow donor (1). It is an attractive alternative source of hematopoietic stem cells to bone marrow or mobilized peripheral blood and is being used increasingly to restore the formation of blood cells not only in patients with hematologic disorders and malignancies, but also those with inherited immunodeficiencies, metabolic diseases (2, 3), and solid tumors (3).

As compared to other sources of hematopoietic stem cells, like peripheral blood and bone marrow, the umbilical cord blood offers numerous logistic and clinical advantages such as: 1) immediate availability of cryopreserved units in public umbilical cord blood banks, and which decrease an average 25-36 days the wait for transplantation as compared to bone marrow, 2) extension of the pool of donors due to the tolerance of up to two mismatches in the Human Leukocyte Antigens (HLA) system, 3) lower frequency and severity of the graft-versus-host disease (GVHD), 4) lower risk of transmission of latent infections such as cytomegalovirus (CMV) and Epstein Barr Virus (EBV), 5) absence of risk to the donor, and 6) higher incidence of rare haplotypes than those found in the records of bone marrow donors (4). Thus, this source of stem cells has been successfully replacing bone marrow and apheresis in transplants, such that in many countries the transplants using umbilical cord blood has outnumbered those with other sources of stem cells.

The first transplantation using umbilical cord blood was performed by Gluckman and colleagues (5), in 1988. The successful use of these cells in transplants brought about the need for storing umbilical cord blood. Therefore, the first public umbilical cord blood bank was established in 1993 by Rubinstein at The New York Blood Center (6). This procedure encouraged the establishment of other umbilical cord banks in various parts of the world, and the number of transplants using cord blood cells has increased remarkably since 1997 (1); until November 2005, according to NetCord, 104,447 umbilical cord blood units had already been stored (www.netcord.org).

Since then, the studies have progressed as to the procedures of collection, processing, characterization, quantification, cultivation, cryopreservation, thawing, and transportation of umbilical cord blood around the world (7).
Some types of stem cells were identified in umbilical cord blood, such as hematopoietic stem cells, mesenchymal stem cells and endothelial progenitor cells. The characterization of the stem cells from the umbilical cord blood units facilitates the understanding of factors affecting the quality and improvement of transplant outcomes (8). For this reason, standards for processing, quantifying, manipulating, cultivation and freezing must be established and followed in order to ensure the minimum characteristics of the unit to be used.

2. General characteristics of hematopoietic stem cells

Hematopoietic tissues contain a small population of primitive and multipotent hematopoietic stem cells. These cells are defined by their ability of self-renewal and proliferation, as well as to differentiate into all of the blood cell lineages, generating committed progenitors of the different myeloid and lymphoid compartments (9, 10, 11). The complexity of this system is enormous, since as many as $10^{10}$ erythrocytes and $10^{8}$-$10^{9}$ white blood cells are produced each hour each day during the lifetime of the individual (12).

A single stem cell has been proposed to be capable of more than 50 cell divisions or doublings and has the capacity to generate up to $10^{15}$ cells, or sufficient cells for up to 60 years (13). The proliferation and differentiation of cells is controlled by a group of proteins called hematopoietic growth factors and interleukins (13, 14). If it could replicate this cell amplification in vitro with hematopoietic growth factors, it might be possible to generate large numbers of cells that could be used for a variety of clinical applications (13).

Besides hematopoietic growth factors, the self-renewal, proliferation, differentiation, homing and mobilization of hematopoietic progenitors are regulated by a complex mechanism involving the bone marrow microenvironment. The adhesion molecules expressed in the hematopoietic progenitors play an important role in these processes. The expression of these molecules has been of particular interest in the studies with umbilical cord blood.

3. Phenotypic Characteristics

3.1. Surface molecules expressed in stem and progenitor cells from umbilical cord blood
The most primitive human hematopoietic progenitor cells have demonstrated expression of CD34, CD45<sup>low</sup>, Thy-1 (CDw90), c-kit receptor (CD117), CD133, and CD164, being negative for CD38, HLA antigens and lineage markers (Lin) (11, 12, 15, 16, 17, 18).

Besides these, several other molecules, including adhesion molecules, have been described as present in the cellular surface of hematopoietic stem cells and will be described below.

### 3.1. CD34

The CD34<sup>+</sup> protein is a surface glycoprotein of 90 to 120 kDa expressed on developmentally early hematopoietic stem and progenitor cells in umbilical cord blood and in bone marrow (10, 19) as well as endothelial cells (20, 21, 22). It has been suggested that this molecule works by regulating the adhesion of the hematopoietic cell to the stroma of the hematopoietic microenvironment (21, 23).

Although the CD34 antigen is the classic marking molecule of hematopoietic stem cells, there is evidence that the progenitors of a yet uncommitted population of stem cells do not express this marker. Depending on the stage of differentiation, a CD34 negative stem cell may generate not only hematopoietic progenitors but also more specific mesenchymal precursors, such as osteoclasts, chondrocytes, myocytes, adipocytes and others. Recent studies have demonstrated the remarkable plasticity of the population of primitive stem cells, comprising cells designed to form the hematopoietic stroma, as well as hematopoietic and mesenchymal progenitors (24).

It has been suggested that CD34 may be a marker of activated stem cells, since CD34<sup>−</sup> (negative) cells in culture originate CD34<sup>+</sup> cells (25). Other studies demonstrate that CD34<sup>+</sup> cells may be reservoirs of CD34<sup>−</sup> cells, showing that the expression of CD34 may be reversible in the hematopoietic stem cells (26).

In several reference centers, the quantification of the CD34 marker has been used to choose a unit of umbilical cord blood. However, the knowledge and standardization of umbilical cord blood CD34<sup>+</sup> cells phenotype is critical since the volume of umbilical cord blood is limited (27).

### 3.1.1. Frequency of hematopoietic stem cells from umbilical cord blood

Parameters commonly used to evaluate an umbilical cord blood unit and predict transplant outcomes have been total nucleated cells and CD34<sup>+</sup> cells counts (28).

Approximately 1% to 3% of total nucleated cells of the bone marrow, including hematopoietic stem cells and endothelial cells, are CD34<sup>+</sup> cells (29-31). In umbilical cord blood, the number of CD34<sup>+</sup> cells is around 1% to 2% among mononuclear cells (33-36). In peripheral blood, the percentage of CD34<sup>+</sup> cells among the total nucleated cells is smaller, ranging from 0.01% to 0.1% (29).
However, studies have shown great variation in the number of hematopoietic stem cells of umbilical cord blood. The number of CD34+ cells among the leukocyte marker, the CD45+ molecule, has already been described as being 0.28 ± 0.15% (37) or 0.4 ± 0.03% of total CD45+ cells, in umbilical cord blood samples in term newborn (38). Among cord blood mononuclear cells, the frequency of CD34+ cells was found to be 1.4% ± 0.9% (36) or 0.36 ± 0.33% (39) with a large variation among samples (range 0.4 to 4.9% and 0.02 to 1.43%, respectively). The absolute number of CD45+ cells in umbilical cord blood of a term newborn has been shown to be about 12 ± 1.3 x 10^6/mL, while the concentration of CD34+ cells is around 5.6 ± 3.9 x 10^4/mL (38). Several studies have estimated that the number of CD34+ cells ranges from 15 to 100 cells per microlitre of umbilical cord blood (37, 40, 41). However, some studies showed a greater variation of these cells, from 22 to 600 CD34+ cells per microlitre of umbilical cord blood (42). These differences may be a result of the heterogeneity proper of umbilical cord blood cells as well as of differences between the techniques used by the various group (43).

These findings show the importance of using standartized methods in the quantification of CD34+ cells of umbilical cord blood.

3.1.1.2. Clinical relevance of the quantification of CD34+ cells

Besides HLA compatibility, the parameter commonly used in choosing an umbilical cord blood unit for a probable successful transplant has been the count of total nucleated cells and CD34+ cells (28). The quantity of total nucleated cells present in the sources of hematopoietic stem cells is of paramount importance for myeloid, lymphoid, and platelet recovery in transplants of hematopoietic stem cells, as well as in post-transplant survival (44).

Nevertheless, as yet there is no consensus about the minimum number necessary for a successful transplant. It has been suggested though that the minimum number of nucleated cells in umbilical cord blood to be used in transplants in order to reduce the time of hematopoietic recovery should be 1.5x10^7 (45) or 2x10^7/kg^2 (4) per kilogram of recipient body weight.

The number of CD34+ cells is also an important issue in hematopoietic stem cells transplants, since it has been suggested to have an correlation between the number of these cells with total nucleated cells and the quantity of CD34+ cells (46). Studies designed to determine the role of the quantity of CD34+ cells show that a precise dose is yet unknown and that this factor may suffer variations depending on the source of hematopoietic stem cells, donor type, relative or non-relative, number of incompatible HLA alleles, and the type of recipient disease (47). Recent works suggest that a minimum of 2x10^5 CD34+ cells per kilogram of patient should be used (4), since high doses of CD34+ cells result in increased myeloid and platelet recovery (48), and other data evidence that a more slow graft "catch" is due to a low number of primitive CD34 cells (49). The "catch" refers to the
ability of infused cells, as they reach the bone marrow, to generate mature cells, detected in the blood flow (50).

3.1.1.3. Quantification of CD34+ cells from umbilical cord blood

In many transplant centers throughout the world, the total number of positive CD34+ cells is used as a parameter when selecting umbilical cord units suitable for transplantation since the adequate quantity of these cells is an important factor for hematopoietic reconstitution (51).

As it is a matter of searching for rare events, given the low frequency of CD34+ cells, standardized, well-established techniques should be used (52). The use of validated protocols, with a proven coefficient of low inter-laboratory variation, is of paramount importance as regards the control of quality in these umbilical cord blood banks. In using a protocol which has less than 10% of inter-laboratory variation for this single-platform technique (49), it is certainly adding quality to the samples stored and which may come to be used by any recipient in need.

The two most widely employed methods of quantification of human umbilical cord blood CD34+ cells are the ISHAGE (International Society of Hematotherapy and Graft Engineering) protocol and the ProCOUNT™ (BD) method (Becton Dickinson) (29).

As a method for standardized analysis of CD34+ cells and use by worldwide banks, Sutherland et al. proposed the ISHAGE guidelines in 1996, currently called ISCT protocol (International Society for Cellular Therapy). This protocol is based on the combination of cell characteristics measured by flow cytometry (29). This technique uses a sequential gating strategy allowing the selection of populations of interest (51), using 4 parameters: size, complexity, CD34 and CD45, evaluated by flow cytometry and able to detect 1 CD34+ cell among 10,000 cells.

However, this protocol has been suffering modifications in order to further improve the techniques of analysis and achieve more accurate quantification. In this way, Gratama and colleagues (53) introduced a marker of cell viability 7AAD (7-Amino Actinomycin D) for determination of the number of viable and inviable CD34+ cells (54). In 2001, Brocklebank and Sparrow (42) described a protocol combining the attributes of the ISHAGE method, use of 7AAD, and use TRUCOUNT tubes, which contain a lyophilized pellet of a known number of fluoresct beads (Figure 1). A reliable rapid method is thus obtained, which employs a single platform and makes it possible to quantify absolute CD34+ cells and assess cell viability using a single technique and the same equipment, thus increasing its sensitivity.
**Figure 1:** Modified ISHAGE gating strategy using TRUCOUNT tubes with FITC-anti-CD45, PE-anti-CD34, and 7-AAD on cord blood. Listmode data were acquired on a FACScan (BD Biosciences) and analyzed with Cell Quest software. Plot 1: Threshold on FL-1 channel; all events collected are shown in this ungated plot. Plot 2: Discrimination of live cells from dead cells. Events falling in R7 are gated out of further analysis. Plot 3: First ISHAGE plot gated on live cells. R1 defines all CD45+ leukocytes and R5 defines the lymphocyte population. Plot 4: Second ISHAGE plot, gated on live CD45+ leukocytes. R2 defines possible CD34+ cells. Plot 5: Third ISHAGE plot, gated on live CD45+CD34+ events. R3 defines clustering of CD34+ events. Plot 6: Fourth ISHAGE plot, gated on live CD45+CD34+ events. R4 defines clustering CD34+ events which have similar light scatter properties and is used to enumerate CD34+ cells. Plot 7: Live lymphocytes. The left side of R4 is drawn to include cells no smaller than a small lymphocytes, and assists to position the exclusion gate in Plot 9. Plot 8: R6 defines TRUCOUNT beads; this plot assists to define the lower limit of CD45 expression of CD34+ cells. Plot 9: Scatter plot. R8 defines an exclusion gate, which removes most of the platelets and debris while allowing TRUCOUNT beads to be collected (42).
It is known that 7AAD identifies dead or apoptotic cells. Many studies in the literature show that 7AAD is very important for the quantification of CD34+ cells in the ISHAGE protocol, since this dye identifies dead CD34+ cells and weakly labels CD34+ cells in apoptosis (55), and are therefore, not contributing to engraftment. It has been shown that the use of 7AAD can decrease by 50% the presence of CD34+ cells per microliter of blood, suggesting that these were unviable cells (49).

However, the use of 7AAD shows controversial results too when protocols using or not using it are compared (56). However, where the samples are processed within 36 hour postpartum, it reduces the possibility of there being dead or apoptotic cells, since the viability of umbilical cord blood cells is around 95% (57-59). Nevertheless, notwithstanding these conflicting results and the detection of unviable cells remaining problematic, there are situations in which the use of 7AAD may be justified and its use remains consensual. It is particularly important in cases where CD34 cells are clinically used, such as in umbilical cord blood banks, the measurement of cell viability through 7AAD is important, since the number of these cells is used to choose an umbilical cord blood sample. Also, in cases where the samples are cryopreserved, as in umbilical cord blood banks, after thawing of samples, or after cell cultivation, whose aim is to transplant frozen or expanded cells, cell viability may be much affected (36, 55, 59, 60). It is thus suggested that 7AAD should be routinely used for quantification of CD34+ cells in the ISHAGE protocol (49).

3.1.2. CD33

The CD33 molecule is expressed in myeloid progenitors (CFU-GEMM, CFU-GM, CFU-G, BFU-E), monocytes/macrophages and in granulocyte precursors. Expression decreases with maturation and differentiation. It is expressed at a low level on mature granulocytes. Expression outside of the hematopoietic system is unknown. The expression of CD33 is a feature of multipotential hematopoietic stem cells, but not of “true stem cells” (www.ncbi.nlm.nih.gov/prow). It has been shown that less than 30% of CD34+ cells express the CD33 marker (10).

3.1.3. CD38

The CD38 molecule is variously expressed by most hematopoietic cells, particularly during early differentiation and cell activation (www.ncbi.nlm.nih.gov/prow). It is expressed, too, in subgroups of CD34+ cells, primitive or activated T and B cells, plasmocytes and thymocytes (61), monocytes, NK cells, and myeloid progenitors (32), as well as in brain, muscle, and kidney cells and other tissues (www.ncbi.nlm.nih.gov/prow). Regarding function, it may play a role in cell activation, proliferation or cell survival (32). It downregulates and upregulates cell activation and proliferation, depending on the cell microenvironment. It is also involved in the adhesion between human lymphocytes and endothelial cells (www.ncbi.nlm.nih.gov/prow).
The most primitive human hematopoietic progenitor cells, CD34+, express little or no express CD38 (11, 12, 15, 16). In this way, expression of the CD38 molecule identifies a CD34+ cell that is already committed, while the CD34+CD38- phenotype identifies a subset of more primitive hematopoietic stem cells (28), with greater ability to generate clones and to allow the expansion of CD34+ cells in culture (62), besides being responsible for the "catch" of the graft in the long run (63). The presence of the CD38 antigen appears to reflect cell activation and differentiation, since cells with the CD34+CD38- phenotype are capable of dividing and proliferating in vitro, for long periods, without undergoing differentiation (64).

About 1% of bone marrow cells express CD34, and generally less than 1% of these cells are CD38-negative. Hence, the frequency of this population is about 1 in 10,000, or even lower. Phenotypic analysis of several cell surface markers reveals that even this rare population is highly heterogeneous. However, it has been observed that the number of CD34+CD38- cells is significantly higher in cord blood than in bone marrow (16±8.8% and 4.7±3% of total CD34+ cells respectively) (65).

On the other hand, controversial results have been published regarding the frequency of CD38- cells among cord blood CD34+ cells. It has been showed that among CD34+ cells 2.6±2.1% (range 0.55–5.57) (36), 3.9±0.9% (38), 11.94±2.09% (17), 13.9% (39), 16±8.8% (65), 34.9±3.4% (66) or 67.9±7.2% (67) are CD38 negative. These findings show the great difficulty in quantifying these events, considered as rare.

It has been demonstrated that phenotype CD34+CD38- characterizes a cell as a candidate of being a "true hematopoietic stem cell". The fact that the frequency of CD34+CD38- cells is greater in the umbilical cord blood (65, 67-69), than in the other two available sources (bone marrow and peripheral blood), might explains the successful clinical of its use in transplants (70) even when low number of cells are used, and candidates these antigens as the predictive parameter for clinical use of umbilical cord blood samples (8, 70), since these cells are responsible for long term graft survival (63).

The great variability and controversial results reported for CD34+CD38- cells in freshly collected cord blood can be explained by the natural heterogeneity of the CD34+ population or by factors that can change the number of the CD34+ cells, for instance differences in the gestational age (48, 71). Alternatively, different volumes of cord blood collected might be responsible for this variation, since it was hypothesised there are more CD34+CD38- cells in the last fraction of the cord and placenta blood than in the first ones (28, 70). In these studies, the authors found in the first fraction 1.61±1.12% CD34+CD38- cells in comparation with the last fraction, where they found 18.98±13.96% of these cells (28). This suggests that the higher the volume collected, the higher the probability of obtaining more residual, immature cells from the placenta.
Although the success of umbilical cord blood cells transplantation is largely related to the number of total nucleated and CD34+ cells, umbilical cord blood CD34+CD38- cells possess high potential of proliferation and expansion of CD34+ (62) cells, suggesting possible advantages concerning the homing and engraftment of more undifferentiated cells (68). The proliferative capacity is also negatively correlated with gestational age (48), corroborating the hypothesis that CD34+CD38- cells are more primitive hematopoietic stem cells with higher clonogenic capacity. On the other hand, it has been suggested that the superiority of using umbilical cord blood in hematopoietic cells transplantation is more related to the ability to generate progenitors than to the frequency of CD34+CD38 cells proper (72).

3.1.4. CD45

Expressed, typically at high levels, on all hematopoietic cells, the CD45 molecule is known as the leukocyte marker. This expression occurs at a higher density on lymphocytes, approximately 10% of the surface area is CD45. While still abundant, the expression is lower on other leukocytes (www.ncbi.nlm.nih.gov/prow).

3.1.5. Thy-1

CD90, or Thy-1, is expressed by hematopoietic stem cells and neurons. This molecule is highly expressed in connective tissue and various fibroblast and stromal cell lines (http://mpr.nci.nih.gov/prow). It has been suggested that Thy-1 is involved in the inhibition of cell proliferation (30). The Thy-1 molecule is expressed on 10-40% of CD34+ cells in bone marrow (http://mpr.nci.nih.gov/prow). Thy-1 co-expression profiles on the cell subsets defined by internal/external CD34 phenotyping are different when comparing bone marrow with cord blood. Although the extCD34+/Thy-1 immunophenotype reportedly highlights a primitive cord blood hematopoietic stem and progenitor cells population, many groups have found that a significant proportion of primitive cord blood mononuclear cells do not express Thy-1 (17).

3.1.6. CD117

The CD117 molecule is the receptor of the stem cells growth factor and it induces his tyrosine kinase activity (32, www.ncbi.nlm.nih.gov/prow). A member of the immunoglobulin superfamily adhesion molecules, this marker is involved in the interactions of CD34⁺ cells with stromal and other cells in the bone marrow, mobilized peripheral blood and umbilical cord blood. This marker has a relevant role in the viability, differentiation and proliferation of hematopoietic stem cells (73, 74).

The expression of c-kit receptor has been reported for characterizing the primitive hematopoietic stem cells (75), since this molecule can be detected in most CD34⁺ cells (16). This marker was detected on the majority of CD34⁺ hematopoietic stem cells, particularly in human umbilical cord blood, where it was found 80.7±8.2% of the CD34⁺ cells, positive for CD117, while on the CD34⁺ cells from bone marrow and peripheral blood the c-kit was found in 72.3±13.1% and 64.2±17%, or lower, of these cells, respectively (16, 76).

The literature findings, however, are still conflicting as to whether these more primitive, hematopoietic stem cells express this molecule in higher (11, 12, 16, 36, 75, 77), or lower levels (78), or do not express it at all (79). The expression of this marker can vary as well according to the subset of hematopoietic stem cells, since its expression among CD34⁻CD38⁺ and CD34⁺CD38⁻ cells can be 80±10% and 56±24% of these cells, respectively, in the umbilical cord blood (36). In evaluating the expression of surface and intracytoplasmic antigens among cells containing intracytoplasmic CD34 (int CD34) yet non-expressed on the surface, 86.36±7.83% of CD34⁺ cells were found be positive for CD117 (17).

Studies showed that cells with CD34⁺CD117low phenotype have been used to describe quiescent progenitor cells, based on the fact that the low c-kit expression on the cell surface might "protect" the cells, preventing it to receive stimuli and differentiate itself, thus characterizing it as a more primitive cell (80). Other studies showed that cells with CD117high phenotype may be ones related to the formation of cell colonies (17).

It has been demonstrated that myeloid progenitors are enumerated in CD34⁺c-kithigh cells and erythroid progenitors are more enriched in CD34⁺c-kitlow cells (81). In contrast, it has been found that erythroid progenitors are highly enriched in mobilized peripheral blood CD34⁺c-kithigh cells, and that CFU-GM is enriched in mobilized peripheral blood CD34⁺c-kit⁺⁻ cells. Primitive progenitors with self-renewal potential may present in the mobilized peripheral blood CD34⁺c-kit⁻ or low cell population (76). It was reported that the human umbilical cord blood-derived CD34⁺c-kitlow cell population contains the majority of cell cycle dormant progenitors and blast cell colony forming cells. The expression of c-kit may therefore be useful in identifying human umbilical cord blood progenitors with long-term engraftment capability (82).

3.1.7. CD133

The CD133, or AC133, molecule is a 120kDa transmembrane glycoprotein, belonging to the family of mucoproteins (18). This marker is expressed on primitive cell populations, such as CD34 hematopoietic stem and progenitor cells, neural and endothelial stem cells, and other primitive cells such as retina and retinoblastoma and developing epithelium. The CD133 expression has also been demonstrated in developing epithelium (neuroepithelium, kidney and gut) in five weeks human
embryos, retina and retinoblastoma cell lines express CD133 antigen and endothelial cell precursors (hemangioblasts). The population of CD133 positive cells contain CD90 (Thy-1) positive, most of the CD117 (c-kit) positive, most of the HLA-DR positive population of progenitors (http://mpr.nci.nih.gov/prow).

The CD133 antigen has been used to characterize more immature hematopoietic stem cells, since in cord blood, the expression of internal CD34 (intCD34+) could be detected on co-expressing CD133+ cells before expression of external CD34 antigen (extCD34+) (17). It is important to observe that intCD34+ cell subsets are consistently and significantly enriched for cells with more primitive phenotypes. It has also been reported that CD133+ cells demonstrated a higher proliferation potential and contain long-term culture-initiating cells (LTC-IC) at a higher frequency than CD34+ cells (17, 83). CD133 antibody has been used for positive selection of hematopoietic stem and progenitor cells for transplantation studies as an alternative to the widely used CD34 (http://mpr.nci.nih.gov/prow).

CD34+CD133+ cells indicate primitive stem cells that are important in repair and regeneration of lesions to an organ, as well migration of stem cells to the site of the lesion. Molecule CD133, expressed in endothelial progenitor cells, contribute to vasculogenesis (84-87). As CD133 populations are known to have the ability to develop into endothelial lineage, it is hypothesised that following cardiac insertion, damaged capillary beds are reseeded by CD133 positive cells that have migrated to the site of injury (17).

3.1.8. FLT3

The Flt3 receptor, or CD135, is a growth factor receptor for early hematopoietic progenitors (http://mpr.nci.nih.gov/prow). FLT3 is expressed on primitive human and murine hematopoietic progenitors cells (11). It enhances hematopoietic cell proliferation and facilitates hematopoietic stem cell mobilization in vivo (88). The class III receptor tyrosine kinase, FLT3 represents an important molecule involved in early steps of hematopoiesis. In cord blood, the majority of CD34+CD117+ (c-kit+), CD34+CD90+ (Thy-1+), and CD34+CD109+ cells coexpress FLT3 (89)

3.1.9. CD164

The molecule CD164 is a 80-to 90-kD transmembrane glycoprotein sialomucins expressed by human CD34 (+) hematopoietic progenitor cells and it have been implicated in cell-to-cell interactions and activations. The CD164 antigen, expressed on early hematopoietic populations, is reported to have a possible function facilitating CD34+ cells to adhere to bone marrow stroma (90). CD164 has been also demonstrated to be highly expressed on strongly positive CD133+ CD38low cells than on those more mature weakly positive CD133+CD38+ cells (17). CD164+ cells represents about 20% of cord blood mononuclear cells and about 60% of cord blood cells coexpressing CD34+CD164+ cells also expressed AC133 (90).

This receptor may play a key role in hematopoiesis by facilitating the adhesion of CD34(+) cells to bone marrow stroma and by negatively regulating CD34(+) hematopoietic progenitor cell growth. It has been reported that the majority of CD34(+) human cord blood cells that were CD38(low/-) or that coexpressed AC133, CD90 (Thy-1), CD117 (c-kit), or CD135 (FLT-3) are CD164+ (91).
3.1.10. CXCR4

CXCR4, or CD184, is the receptor for the stromal-derived factor (SDF-1) (88, 92-94). CXCR4 is a common marker of hematopoietic, endothelial, neural, muscle, and liver stem cells. SDF-1, the CXCR-4 ligand, is secreted in various organs to which circulating stem cells are chemoattracted and "home/reside." Circulating stem cells may compete for common tissue-specific niches with the result that stem cells committed to other tissues may be detected in various organs. Furthermore, CXCR4 has recently been reported to be present on primordial germ cells, neural stem cells and retinal pigment epithelial stem cells as well as liver oval stem cells. More importantly, CXCR4 is functional on all of these cells and CXCR4-positive tissue-committed stem cells respond by chemotaxis to an SDF-1 gradient (95).

In a study comparing the CXCR-4 expression among different sources of hematopoietic stem cells like peripheral blood, bone marrow and fetal blood, the frequency of this marker in CD34+ cells from cord blood is highest (96). About 90% of CD34-cord blood cells are positive for this marker (96, 97). Because the CXCR-4 receptor is expressed in umbilical cord hematopoietic stem/progenitor cells, it plays a crucial role in the homing of these cord cells to the bone marrow microenvironment (95).

3.1.10.1. The importance of Homing

Homing is the first and a rapid process in which circulating hematopoietic cells actively cross the blood/bone marrow endothelium barrier and lodge at least transiently in the bone marrow compartment by activation of adhesion interactions prior to their proliferation. Stem cells also home to other organs, especially in response to stress signals, transmitted in response to alarm situation (98).

The ligand for CXCR4, SDF-1, is secreted by bone marrow fibroblast and plays an important role in the homing/retention of hematopoietic stem cells in the bone marrow microenvironment. SDF-1, however, is also secreted in several other organs and, for example, is detectable in heart and skeletal muscles, liver, neural tissue and kidney. The secretion of SDF-1 increases during muscle ischemia, toxic liver damage or total body irradiation. Thus, it has been hypothesized that SDF-1 plays an important role in heart regeneration by attracting CXCR4-positive muscle stem cells. On the other hand, SDF-1 secreted during tissue damage may play an important role in directing tissue-committed stem cells necessary for organ/tissue regeneration (95).

When there is tissue damage, CD34+CXCR4+ cells migrate to the site of the lesion, attracted by the secretion of the SDF-1 receptor, the ligand of receptor CXCR4. Niches of SDF-1 are found in injured organs and are released during tissue damage. It is well-known that the organization of cells niches have a key role in the normal regulation of stem cell differentiation and regeneration (99). Thus, CXCR4+ cells are important in the regeneration of injured organs, indicating the regenerative ability of stem cells (94, 97). The bone marrow and the skeletal muscle also contain a population of CXCR4 cells which express specific genes for muscle progenitor cells and which can be mobilized to the peripheral blood. Thus, SDF-1 is an important factor that influences the mobilization of bone...
marrow cells (100). After myocardial infarction, for example, the CXCR4 cells are mobilized from bone marrow into the peripheral blood and chemoattracted to the infarcted myocardium (101).

These findings show the importance of CXCR4 in homing and, since the cord blood cells are rich in these molecules, this is an advantage of using the umbilical cord blood cells.

3.2. Other markers and adhesion molecules expressed in hematopoietic stem and progenitor cells from umbilical cord blood.

Adhesion molecules play a role in the migration of hematopoietic progenitor cells and regulation of hematopoiesis (102). There is evidence that cord blood, bone marrow and peripheral blood-derived hematopoietic stem cells are highly heterogeneous for a number of antigens useful for hematopoietic stem cells enumeration by flow cytometry (103). Cell adhesion molecules are highly expressed in both human umbilical cord blood and bone marrow CD34⁺CD38⁺ cells. Since the expression of such molecules has been related to the repopulating capacity of hematopoietic progenitor cells, there is a possible advantage in homing and engraftment of more undifferentiated human umbilical cord blood as opposed to bone marrow hematopoietic progenitor cells (65). Since the blood release of hematopoietic progenitor cells is probably due to a perturbation of the adhesive interactions between these cells and the expression on CD34⁺ hematopoietic progenitor cells found in the three hemopoietic compartments evaluated can lead to new knowledge about the mobilization kinetics in which the adhesion molecules are involved (16).

The adhesion molecules allow interaction with various regulatory elements present in the microenvironment, which include stromal cells, molecules of the extracellular matrix, and soluble regulatory factors such as cytokines or growth and cell differentiation factors (104). The hematopoietic stem and progenitor cells, most of which expressing the CD34 antigen, have multiple adhesion receptors. These receptors allow binding of stem or progenitor cells to the components of the extracellular matrix within the medullary sinusoids, thus facilitating its homing in the bone marrow and promoting a close cell-cell contact necessary for cell survival and cell proliferation regulation. There are several adhesion receptors and their ligands, present in stem and progenitor cells and in components of the hematopoietic microenvironment (61). Some of the subgroups of receptors are: integrins (such as CD11c and CD49e), molecules of the superfamily of immunoglobulins (such as CD31 and CD117), lectins or selectins (such as CD62L), sialomucins (such as CD34), CD38, among others.

3.2.1. CD11c

The CD11c molecule is an integrin of the leukocyte surface found in em macrófagos, NK cells, subpopulation of T and B cells (www.ncbi.nlm.nih.gov/prow), monocytes and polymorphonuclear
neutrophils (32). This adhesion molecule has a role in the linkage to receptors on the stimulated endothelium (www.ncbi.nlm.nih.gov/prow).

It has been shown that the expression of CD11c is rare in CD34+ cells of both human umbilical cord blood (36, 105) and bone marrow (106).

3.2.2. CD31

Also known as PECAM-1 (platelet endothelial cell adhesion molecule-1) the molecule is present in myeloid cells, leukocyte and their precursors, endothelial cells, CD34+ cells, monocytes, neutrophils (32, 61), platelets, NK cells, T cells subgroups, but not on circulating B cells (www.ncbi.nlm.nih.gov/prow). CD31 binding activates leukocyte integrins (32). This molecule is involved with the adhesion between cells such as endothelial and leucocytes (www.ncbi.nlm.nih.gov/prow), as well as with the interaction between hematopoietic cells and extracellular matrix components in bone marrow (107).

Several reports have shown high expression of CD31 on bone marrow (102, 106) and umbilical cord blood (36, 110) CD34+ cells.

3.2.3. CD49e

The CD49e molecule corresponds to the alpha chain of the VLA-5 integrin, (www.ncbi.nlm.nih.gov/prow) (32, 61), and it is expressed on cellular surface of thymocytes, T cells, monocytes, activated platelets and primitive B cells (32) and in CD34+ cells (61). VLA-5 is strongly involved in the binding of bone marrow progenitor cells to extracellular matrix components (108).

It is interesting that different reports have conflicting results regarding this molecule. It was already shown that all CD34+ cells in normal bone marrow expressed CD49e, while cord blood and mobilized CD34+ cells had a lower expression of this molecule than bone marrow CD34+ cells (106). Other studies showed opposite results (65) or that cord blood CD34+ cells have a remarkably similar (109, 110) expression of VLA-5 on bone marrow CD34+ cells. By studying the subpopulation of CD34+ cells, it has already been shown a large number of CD34+CD38- and CD34+CD38+ cells positive for CD49e, before and after culture of umbilical cord blood-derived CD34+ cells with some combinations of hematopoietic growth factors (36).

3.2.4. CD61

The CD61 molecule is the beta 3 integrin chain, also called GPIlb/IIIa (www.ncbi.nlm.nih.gov/prow). It is present in platelets, megakaryocytes, monocytes, macrophages and endothelial cells whose function is to facilitate platelet aggregation (32).

CD61 has been observed in small levels on human umbilical cord blood CD34+ cells, with less (27, 36) or around than 20% (28) of these cells positive for CD61.
3.2.5. CD62L

The CD62L molecule is also called L-selectina, LAM-1 (leukocyte adhesion molecule-1) and LECAM-1 (32, 61, 111, 112, www.ncbi.nlm.nih.gov/prow) and it is present in T and B cells, monocytes, neutrophils, thymocytes, eosinophils, basophils, erythroid and myeloid progenitor and NK cells (32, www.ncbi.nlm.nih.gov/prow). The molecule is also present in a few lymphocytes of the spleen and bone marrow and in myeloid cells of the bone marrow, as well as in certain hematopoietic malignant cells (www.ncbi.nlm.nih.gov/prow).

L-selectin takes part in the homing of lymphocytes and facilitates cell binding to the endothelium at inflammatory sites (32). This molecule is involved in the homing of CD34+ cells after peripheral blood mononuclear cell transplantation (105) and it is suggested that it can increase the clonogenic capacity of CD34+ cells (113).

The majority of the CD34+ cells also had CD62L on the surface membrane. Cord blood and mobilized blood CD34+ have been shown to present a higher expression of CD62L than bone marrow CD34+ cells (106). However, the results are controversial, since in other studies the expression of this molecule seems to be similar (109) or smaller (65) in the stem cells of umbilical cord blood than in stem cells of the bone marrow. It has been showed that CD62L is more expressed, also, in cord blood that in bone marrow CD34+CD38−subset, suggesting a possible advantage in homing and engraftment (65, 68). The great heterogeneity of positive cells in fresh samples as well as small differences after culture, in CD62L molecules could be explained by the natural heterogeneity of CD34+ cells or, perhaps, differences in gestational age, since was showed that CD62L on CD34+ stem and progenitor cells in umbilical cord blood change during gestation (114).

3.2.6. HLA-DR

The HLA molecule Class II is expressed in monocytes, macrophages and lymphocytes, and its function is the presentation of exogen antigens to Th lymphocytes (CD4). HLA-DR is expressed in the majority of human umbilical cord blood (27, 28) and peripheral blood CD34+ cells (27, 76). Some studies showed that the coexpression of CD34 with HLA-DR was not significantly different in human umbilical cord blood and bone marrow (respectively, 86.3±2.7% and 92.7±5.1%) (67), while other showed that among mononuclear cells, CD11+HLA-DR+ cell frequencies did not differ significantly among the three hematopoietic compartments (69).

A great variation was also shown in the expression of HLA-DR molecule, in fresh or cultivated cells. This finding may be ascribed to the heterogeneity of CD34+ cells or differences in gestational age (114). Fetal liver cells, for instance, have been shown to present lower proportions of CD34+HLA-DR- than human umbilical cord blood, showing that the composition of fetal leukocytes changes during development and with gestational age (115). The frequency of HLA-DR-positive cells is a little
higher among CD34⁺CD38⁺ than CD34⁺CD38⁻ cells. These findings support the hypothesis that these molecules are more expressed in more differentiated cells (36).

3.3. Conflicting results in the expression of surface molecules in hematopoietic stem cells

The controversial results presented in the literature about the co-expression of markers in CD34⁺ cells of umbilical cord blood probably reflect the phenotypic and functional heterogeneity of the CD34⁺ cell population. However, it is known that the handling of hematopoietic stem cells, as well as their freezing, for example, changes the distribution of such surface molecules as CD34 (116). The mobilization induced by cytokines can also alter the profile of markers, especially adhesion molecules, in CD34⁺ cells of the bone marrow (117). Finally, it is known that the adhesion molecules and their receptors present interaction among one another and even functional overlay (118).

4. Culture of hematopoietic stem cells from umbilical cord blood

Clearly, the umbilical cord blood is an excellent source of stem cells and its clinical utilization has increased in recent years, even for disorders other than those usually treated with bone marrow transplantation. One problem, however, is that the number of hematopoietic stem cells in umbilical cord blood samples often is limited (3). Identification of conditions that support the self-renewal and expansion of human hematopoietic stem cells remains a major goal of experimental and clinical hematology (119). The expansion of human stem cells ex vivo will likely have important applications in transplantation, stem cell marking, and gene therapy (15, 120, 121).

Because the volume of umbilical cord blood is a limiting factor for the number of stem cells (1, 13), the number of hematopoietic progenitors and stem cells in cord blood is enough to support the bone marrow engraftment in children, but usually it is not enough to successfully engraft an adult (122). Of the 3,942 umbilical cord blood units transplanted from the Netcord Network until November 2005, 2,399 were transplanted in children and 1,528 in adults (www.netcord.org).

The possibility of increasing the dose of cells to be used in the transplantation of human hematopoietic stem cells is very important since the efficacy of cord blood transplantation is limited by the low cell dose available (9, 119, 123, 124, 125, 126, 127). Low cell doses at transplant are
correlated with delayed engraftment, prolonged neutropenia and thrombocytopenia and elevated risk of graft failure. To potentially improve the efficacy of umbilical cord blood transplantation, approaches have been taken to increase the cell dose available. One approach is the transplantation of multiple cord units, another the use of ex vivo expansion (128).

Transplantation of multiple UCB units could be a strategy to overcome cell dose limitations (129). Nevertheless, to find a HLA match in two umbilical cord blood units could be difficult. Ex vivo expansion of hematopoietic stem cells was suggested as the best way of overcoming problems caused by limited hematopoietic cell number for cord blood transplantation (130, 131). As a result, the expansion of human stem cells will have important clinical applications, because their ex-vivo expansion might be required to successfully engraft an adult (122).

Therefore, two important aspects of the biology of ex-vivo expanded cells relate to cultured cells: either maintaining their self-renewal capacity and multilineage differentiation potential, or improving their short-term engraftment ability when transplanted into myeloblated recipients. Several growth factor combinations have been tested to identify suitable culture conditions to induce expansion of primitive stem cells. So far, only a few studies have shown that primitive non-obese diabetic severe combined immunodeficient (NOD/SCID) mouse repopulation stem cells from cord blood can be expanded (a few or several-fold) after in vitro culture (122).

Currently, the umbilical cord blood cells ex vivo expansion processes include: (1) liquid expansion: CD34+ or CD133+ cells are selected and cultured in medium containing factors targeting the proliferation and self-renewal of primitive hematopoietic progenitors; (2) co-culture expansion: unmanipulated cord blood cells are cultured with stromal components of the hematopoietic microenvironment, specifically mesenchymal stem cells, in medium containing growth factors; and (3) continuous perfusion: cord blood hematopoietic progenitors cells are cultured with growth factors in ‘bioreactors’ rather than in static cultures. Ultimately, the goal of ex vivo expansion is to increase the available dose of the cord blood cells responsible for successful engraftment, thereby reducing the time to engraftment and reducing the risk of graft failure (128).

Countless are the factors regulating hematopoiesis. Hematopoietic growth factors are soluble factors influencing the growth or differentiation of hematopoietic progenitor cells. These factors can act directly or indirectly on the cells, binding to cell receptors (14). The interaction of these factors and their receptors on cell membranes is an important mechanism of regulation of survival, proliferation and differentiation of hematopoietic cells (78).

The hematopoietic growth factors are the so-called: colony-stimulating factors (CSFs) (such as GM-CSF, G-CSF and M-CSF); steel factor (SF) or stem cell factor (SCF) or kit ligand (KL); erythropoietin (EPO); thrombopoietin (TPO); tumor necrosis factor (TNF); FLT-3 ligand or FL; interleukins (IL) and others (11, 14). The proliferation and differentiation of hematopoietic stem cells is controlled not only by soluble growth factors, but also by adhesion to stromal cells and matrix molecules (112).

Several culture systems were developed to try to expand hematopoietic stem cells (9, 36, 123, 124, 132). According to literature, several different combinations of growth factors have been used. Some studies showed the differential ability of combination of some growth factors like FLT-3,
TPO, KL, GM-CSF, IL-3 and IL-6, to support different stages of hematopoiesis in long term cultures of progenitor cells from human umbilical cord blood (9, 18, 133).

Several studies showed the effects of thrombopoietin alone in culture, where it can stimulate the early proliferation, survival (125) or differentiation of progenitor cell in cord blood (126) or bone marrow (127). TPO is a primary regulator of megakaryocyte and platelet production and might also play an important role in early hematopoiesis (15). It is an important cytokine in the early proliferation of human primitive as well as committed haematopoietic progenitors, and in the ex vivo manipulation of human haematopoietic progenitors (125). TPO has also been observed to suppress apoptosis of CD34^+CD38^− cells in culture, showing a potential role in maintaining quiescent primitive human progenitor cells viable (134). In studies using a combination of growth factors with and without TPO, a significant expansion of CD34^+ cells from umbilical cord blood and neonatal blood to early and committed progenitors was shown, in the presence of this factor (135).

SCF or KL, also called mast cell growth factor, stimulates the survival and growth of primitive stem cells in synergy with several factors (11, 74).

FLT3 ligand or FL co-stimulates the multipotent stem cells, especially with thrombopoietin and kit-ligand. It stimulates the generation of dendritic cells and induces regression of tumors in vivo (11). It is able to induce proliferation of CD34^+CD38^− cells that are nonresponsive to other early acting cytokines and to improve the maintenance of progenitors in vitro (124).

It has already been shown that, although TPO alone can stimulate limited clonal growth, it synergizes with SCF, FL, or IL-3 to potently enhance clonogenic growth (15). Several published studies have shown the increase of this cell population, even with TPO alone. However, in many studies using umbilical cord blood, the expansion of nonadherent cells was greater with TPO, FL and SCF than TPO and FL, and greater in this combination than with TPO alone (9, 120), especially in the sense of achieving expansion of CD34^+CD38^− cells in vitro (136) based on the proliferative potential of these cells present in the umbilical cord blood (36, 72).

Some cell surface molecules can change their expression after cultivation with growth factors. For example, the number of CD62L-positive CD34^+CD38^− and CD34^+CD38^+ cells and the CD62L expression on these cells increase during short-term culture with TPO, FL and SCF (36). It has already been showed that a short exposure to cytokines increases L-selectin expression in the more differentiated hematopoietic progenitors, CD34^+CD38^− cells which could improve their homing in a transplant setting. After transplantation of hematopoietic stem cells, adhesion molecules play a major role in the multistep process of engraftment in which L-selectin is suggested to be of relevance (36, 65). The expression of c-kit (CD117) on CD34^+CD38^− and CD34^+CD38^+ cells decrease, in some studies, after culture with TPO, FL and SCF. Culture of CD34^− cells with TPO, FL and SCF thus significantly increases the number of candidate stem cells with the CD34^+CD38^− (c-kit) phenotype. On the other hand, the down-regulation of c-kit may be due to the presence of SCF in the growth factor combination, since this factor was essential to expand CD34^+CD38^− cells. In the same growth factor combination, the number of cells positive for HLA-DR and the intensity of fluorescence increased in both CD34^+CD38^− and CD34^+CD38^+ cells (Figure 2) (36).
A number of cultivation strategies have been tested for cell expansion. A stroma-free culture with FL, SCF, and TPO allows the maintenance and expansion for several weeks of a cord blood CD34+ cell population capable of multilineage and long-lasting hematopoietic repopulation in non-obese diabetic/severe combined immudeficient (NOD/SCID) mice. Moreover, a long-lasting severe post-transplant trombocytopenia is often observed even in pediatric patients (122).

Selected CD34+ cells, after a 4-week expansion with FL, SCF and TPO, appear to be more efficient in megakaryocyte engraftment than the same number of unmanipulated cells (122). In a comparative study carried out with two groups of irradiated NOD-SCID mice transplanted with expanded and nonexpanded cells from the same umbilical cord blood, the bone marrow was analysed for the presence of human cells. Both groups of mice showed successful engraftment and the cell population obtained after 12 days expansion consisted mainly of myeloid and megakaryocytic progenitors (137).

In many studies cells are cultured with fetal calf serum or pooled human serum (9). However, for clinical use, cell expansion in the absence of serum is a clear advantage. In trials performed with mice receiving human stem cells expanded in serum-free medium with a combination of three (FL, TPO, SCF) or four (FL, TPO, SCF, IL6) growth factors was compared with the results obtained using fetal calf serum (FCS) or human serum (HS). The engraftment of human cells in mice was higher for serum-replete than for serum-free expanded cells. Nevertheless, serum-free cultured cells were also able to engraft both marrow and spleen in all animals. In addition, engrafted human cells still maintained clonogenic ability. With SCF, FL, TPO +/- IL6 it is possible to expand haemopoietic progenitor cells in a serum-free medium (133). It is believed that serum-free medium allows a better control of the role that individual cytokines and their combination have on cell growth and differentiation (36). Compared with serum-replete cultures, the absolute number of clonogenic cells and in vivo repopulating cells is lower. Although the degree of expansion remains significant, a clinical trial still needs to be carried out to address the question of whether this expansion might be useful in reducing post-transplant aplasia (133).

Another important factor influencing the efficiency and practicality of umbilical cord blood cell cultivation is the choice of length of cell cultivation. Some studies use long-term cultures, while other, short-term cultures. While long-term cultures allow the expansion of a greater number of cells, in short-term culture the expansion is lower. On the other hand, in the face of the need for transplanting in a patient with cells from umbilical cord blood, the expansion of CD34+CD38- cells within a short period of time could be better for the necessity of having available cells rapidly for transplants (36).

Finally, another strategy that has been studied, particularly for adult patients where the amount of umbilical cord blood cells would not be enough for the transplant, is the autologous transplant concomitantly with the use of umbilical cord blood cells, whether expanded or not. Therefore, it is fundamental that short-time culture systems are perfected so that more patients can benefit from the transplantation of these cells.
Figure 2: Frequency of cells positive for CD62L/PE, CD117/PE and HLA-DR/PE among CD34⁺CD38⁻ and CD34⁺CD38⁺ cells on day 0 and after 7 days culture with TPO+FL+SCF, serum free. TPO: thrombopoietin; FL: FLT-3 ligand; SCF: stem cell factor. PE: Fluorochrome phycoerythrin. M1: Isotype control; M2: cells with regular fluorescence; M3: cells with bright fluorescence (36).
5. Physiological, hematological and immunophenotypical correlations of umbilical cord blood

Many factors can influence the concentration of progenitor and stem cells in umbilical cord blood.

The quantity of total nucleated cells is correlated with the percentage value of CD34+ cells per microliter of blood as well as there is correlation between collected volume and number of CD34+ cells both per microliter of umbilical cord blood and in the percentage of these cells among CD45+ ones (46, 138).

It is known that higher volume of blood is collected with the placenta still in the uterus (139, 140, 141). Nevertheless, this procedure is not performed in public umbilical cord blood banks collections. It was observed, also, that high volume samples are correlated with high doses of total nucleated cells, CD34+ cells and colony forming units of granulocytes and macrophages (CFU-GM) (7).

In addition, factors like newborn and placental weight (141, 142), longer umbilical cord, cesarean section and advanced gestational age can influence the volume of collected blood and the number of total nucleated cells (142) (Figure 3 A and B). The volume collected could be larger, also, according the effect of “upper” and “lower” positions of the term neonates, vaginally delivered, increasing the progenitor cell (CD34+) content of the umbilical cord blood (143). Other findings can also explain the variability of CD34+ frequency among umbilical cord blood samples. Longer duration stress (a prolonged first stage of labor) of the infant during delivery, for instance, demonstrated increased numbers of nucleated cells, granulocytes, CD34+ cells, and hematopoietic progenitor cells in umbilical cord blood from children with lower venous pH (144).

Also were found positive correlations of advanced gestational age with volume (145) and number of CD34+ cells (46) (Figure 3 C and D). The quantity of CD34+ cells per microliter of umbilical cord blood is inversely proportional to gestational age (10, 43, 48) (Figure 3 E). Yet, if on the one hand the umbilical cord blood of prematures presents a higher quantity of CD34+ cells per microliter, the collected volume is lower than that obtained from term fetuses, because the more advanced the gestational age, the higher the placental weight and the higher the volume of blood that can be collected (142).

Although some form of linear correlation between total nucleated cell and CD34+ cells in cord blood has been reported, within groups of samples with similar total nucleated cells counts a high degree of variation (at times exceeding 10-fold) in CD34+ cells is observed (36, 43) (Figure 3 F). Different explanations have been given to the variability found on the frequency of CD34+ cells in umbilical cord blood. There is evidence that, although the CD34 population is a reliable indicator of the progenitor potential of human umbilical cord blood it is nevertheless heterogeneous in nature. On the other hand, these heterogeneous results can reflect differences in the sensitivity of the methods employed by the different groups. Besides gestational age, CD34+ hematopoietic stem cells have also
been shown to vary with mode of delivery and positioning of the delivered neonate after delivery, inasmuch as these factors can affect the volume of collected blood (43, 143).

The frequency of CD34+ cells was shown to decline linearly with gestation age, being significantly higher in the early gestational age than term gestation fetuses (10, 48, 71, 146, 147, 148), decreasing rapidly in the peripheral blood of neonates soon after birth (149). In fetal liver, also, there seems to be a strong and highly significant inverse correlation between CD34+ cells (as a proportion of total leukocytes) and gestational age (115). The proliferative capacity also shows an inverse correlation with gestational age (48).

Some studies report that the number of CD34 cells per microliter of blood is significantly higher in samples coming from cesarean deliveries, due to the higher volume collected (150). On the other hand, in other studies, it did not observe any association of cesarean delivery with number of total nucleated cells, CD34+ cells (46, 145, 151) and CD34+CD38- cells (47). It has also been shown a correlation between the number of erythroblasts with CD34+ cells and CD34+CD38- (46).

With regard to the percentage of CD34+CD38- cells, as previously mentioned there is great data disagreement, which may be accounted for by the difficulty in analyzing such rare events. Moreover, the factors affecting the number of CD34+ cells might also influence the number of CD34+CD38- cells in the umbilical cord blood. The variation in relation to gestational age has already been reported as a factor that is inversely proportional to the number of CD34+CD38- cells in cord blood (48, 71). Though the relation between ethnic origin and quantity of these cells was little investigated so far, it has been found that CD34+CD38- subsets were significantly lower in African American and Asian persons compared to Caucasian and Hispanic persons (46).

It was also shown that cells presenting CD34+CD117 phenotype, or low expression on the surface, appear to vary with the volume obtained, as well as with the presence of total nucleated cells and CD34+ and CD34+CD38- cells. In addition, it was already found negative correlations concerning the relative presence of CD34+CD117- cells among the CD34+ cells population with the parameters volume, total leukocytes, number of CD34+ cells per microliter of umbilical cord blood, and percentage of CD34+CD38- cells. On the other hand, the same parameters presented positive correlations with CD34+CD117+ (Figure 3: G, H, I and J) (152).

Therefore, several factors may influence total nucleated cells and CD34+ cells counts and, hence, the success of a transplant. Procedures such as clamping the umbilical cord as closely as possible to the infant's body, in order to obtain a longer cord, and collecting cord blood as promptly as possible after birth, surely provide higher collected volume (142). In addition, it is suggest that a shorter time interval between collection and handling of the sample (cell counting) can increase the concentration of CD34+ cells (138). Measures like these may be highly valuable in the acquisition and storage of samples with higher quality standards, thus avoiding that such a rich material with so much proliferative potential will not be used to the fullest.
As the volume of collected umbilical cord blood directly affects the quantity of CD34+ cells per microliter of blood and possibly the quantity of total nucleated cells, some measures must be taken at the moment of collection in order to increase the collected volume. These measures include the advice that this procedure must be performed by individuals trained to this end, because the longer it takes from the removal of the placenta and cord, the smaller the yield obtained and, hence, the lower the number of CD34+ and total nucleated cells.
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Figure 3: Correlation between physiological, hematological and immunophenotypical parameters. Significance by Pearson's correlation between the collected volume of umbilical cord blood (UCB) (mL) and (A) the newborn weight (g) \( (r = 0.49, p<0.01) \); (B) the placental weight (g) \( (r = 0.296, p<0.05) \); (C) the absolute number of CD34$^+$ cells per microliter of UCB \( (r = 0.29, p<0.05) \); and (D) the relative number of CD34$^+$ cells among CD45$^+$ cells \( (r = 0.36, p<0.01) \). Correlation of CD34$^+$ cells per microliter of UCB with (E) the gestational age (weeks), with a negative correlation \( (r = -0.33, p<0.01) \); and (F) the absolute number of total leukocytes, \( (r = 0.32, p<0.05) \). A significant positive correlation between the relative number of CD34$^+$CD117$^-$ cells and (G) the volume of UCB \( (r = 0.41, p<0.01) \); (H) the CD34$^+$ cells \( (r = 0.41, p<0.01) \); (I) the leukocytes, \( (r = 0.45, p<0.01) \); and (J) the CD34$^+$CD38$^-$ cells \( (r = 0.29, p<0.01) \) \[152\].

6. Clinical importance of the quantification of hematopoietic stem cells in the umbilical cord blood
Though the umbilical cord blood constitutes a very rich source of hematopoietic cells that can thus be used to reconstitute the blood system (153), an accurate quantification of stem cells in the umbilical cord blood is crucial.

At present, an increasing number of cases are being studied in order to compare the use of umbilical cord blood with other sources of hematopoietic stem cells, particularly the bone marrow, as regards the "catch" potential, GVHD induction, immune reconstitution, effect of graft against the tumor, patient survival, in relation to the dose used and the number of incompatible HLA alleles. As main advantage of umbilical cord blood we can point the rapid availability of the unit, which once located can be promptly used, thus reducing approximately the time in searching for a marrow donor from 4 months to around 13.5 days in searching for a unit of donated umbilical cord blood (44, 154, 155).

Doses above $3 \times 10^7$ total nucleated cells (TNCs)/Kg appear to compensate the negative effect of 1 incompatible HLA allele (156). The risk of developing acute and chronic GVHD in recipients of umbilical cord blood with up to 2 incompatible HLA alleles is similar the that of receiving a bone marrow from an identical HLA donor (154), and the disease-free survival time turned out to be similar (157). However, though the umbilical cord blood constitutes a good alternative to be used in allogeneic transplantation, the number of immature cells is limited by blood volume that is possible to collect, which can bring about a delay in engraftment "catch", particularly in adult patients.

However, the umbilical cord blood can be used as an alternative in adult patients when an identical bone marrow donor is not available (158), since in a study of 68 adult patients receiving transplant of umbilical cord blood, a mean $1.6 \times 10^7$ TNCs and $1.2 \times 10^5$ CD34+ cells per kilogram of the recipient's body weight were enough to ensure engraftment (159). Another alternative would be the utilization of more than one umbilical cord blood unit to increase engraftment "catch". This technique can provide for fast hematopoietic recovery and total chimerism of one of the units at day 100 post transplantation, i.e., at least 90% of donor cells present in the recipient, confirming "engraftment" catch (44).

Moreover, it is suggested that umbilical cord blood cells, when transplanted, possess a reduced potential for homing towards the bone marrow, as compared to more mature cells. However, this difference is compensated by the fact that the umbilical cord blood cells are more easily maintained in their immature state, guaranteeing the levels of progenitor cells for longer, as compared to bone marrow transplantation (98). A more rapid engraftment and better graft survival are obtained when the cord blood provides at least $2.0 \times 10^7$ TNCs and $2.0 \times 10^5$ CD34+ cells per kilogram of the recipient's body weight (4).

It is known that the number of TNCs and the number of HLA mismatches interact in the engraftment and in the frequency of graft-versus-host disease (GVHD), and a higher number of TNCs might be thought to partially suppress the negative impact of HLA mismatches. However, this hypothesis has not yet been totally proven. A higher number of TNCs and a lower number of HLA
mismatches were correlated with a higher likelihood of engraftment. On the other hand, higher HLA mismatch is related to higher incidence of GVHD grades III-IV and a lower incidence of relapse of acute leukemias, evidencing the graft effect against leukemia. Thus, the choice of a good unit of umbilical cord blood to be used must be based on the number of TNCs and on the number of compatible HLA alleles (160).

So, the increasing use of umbilical cord blood cells makes it necessary to expand the public bank network for storage of these cells, since the more units stored the greater the ethnic diversity that can be achieved, eventually permitting the identification of units which also contemplate racial minorities with all their haplotypes (40).

On this account, it is crucial that further research must be done to increase our knowledge of the biology, quantification and factors that may affect umbilical cord blood collections, for each day new results have shown us that we are faced with a material which has great proliferative capacity and from which, according to recent studies, cells with embryonic characteristics have already been obtained (161). With this, research has shown that the umbilical cord blood may in the future be a source of stem cells to treat not only hematological disorders but also several other diseases, since investigations into the plasticity of stem cells have demonstrated the importance of these cells in restoring various other organs. Thus, a better understanding of these cells may generate outstanding findings, highly useful for preserving life, given that hitherto many questions remain unanswered, such as: if we have to choose between a unit with more nucleated cells ($6 \times 10^7$) and one HLA incompatible allele, and another with less cells ($4 \times 10^7$) but no HLA mismatch, what can be best for the patient? (160). Although some studies show that the best option is to use the first sample (156, 160), since the risk of developing acute and chronic GVHD in umbilical cord blood recipients with up to two HLA incompatible alleles is similar to that of a recipient of bone marrow from an identical HLA donor (154), further studies must be performed. Doubts like this may be minimized as the biological properties of these cells are investigated and integrated into the issue that these umbilical cord blood cells do include cells that are immature and highly capable of dividing before differentiating themselves, which may be crucial in evaluating transplant outcomes.

7. Cord Blood of Preterm Neonates
8. Conclusions

Stem cells expressing CD34, CD133 (17) and CXCR-4 (101), and others markers, have been utilized in hematopoietic transplant regiments not only in bone marrow transplant, but also used for cardiac therapeutic procedures improving blood perfusion in cardiac muscle and in other therapeutic procedures. The success of the initial results of the use of stem cells in the treatment of diseases other than those of hematologic origin has opened the possibility of new therapies based on the use of stem cells from umbilical cord blood, which has, among many other advantages, that of having greater donor availability. It is estimated that, as well as widening the clinical use of stem for hematological and cardiac diseases, an extensive variety of diseases, such as auto-immune and neurodegenerative diseases, among others, will be treatable, in the near future, with cells obtained from human umbilical cord blood. Thus, it is of great importance that knowledge of the biology and plasticity of the stem cells present in cord blood is expanded, with the aim of widening its clinical use, making it possible to develop new therapeutic procedures, including gene therapy. It is also necessary to expand the number of public umbilical blood banks in order to increase the supply of units of donated umbilical cord blood, so that, in the future, the cells they possess can be made available for the treatment of a number of serious or currently incurable diseases that affect so many patients.
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Cord Blood Stem Cells-The Basic Science

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Cord blood stem cells have been transplanted since 1988 (1) and are now accepted as an acceptable source of stem cells for transplant in a range of haematological diseases (2) and in the repair of the bone marrow following high dose chemotherapy for cancer. The basis of these applications is that cord blood contains CD34+ myeloid progenitor cells (3) which upon transplantation achieve long-term, stable bone marrow reconstitution (4). To date there have been over 6,000 transplants of cord blood stem cells in the treatment of 45 different diseases (5) and the future can only see an increase in the use of cord blood stem cells in the treatment of a wide range of diseases. The advantages in the use of cord blood as a source of stem cells for transplant are:

- Ease of procurement, processing and storage
- No risk to donors
- Reduced risk of transmitting infection
- Immediate availability of cryopreserved units
- Acceptable partial HLA mismatches (4/6 HLA match)

In addition cord blood stem cells do not carry any of the legal, moral, ethical or religious objections associated with the use of embryonic stem cells.

The current disadvantages in the use of cord blood stem cells as a source of stem cells for transplant are:

• The limited number of haematopoietic stem cells in a cord blood unit which may lead to failed or delayed haematopoietic reconstitution or restricted use in adults
• Possible abnormalities in cord blood stem cells, e.g. early malignant mutations, which may have an effect on recipients
• It is not possible to collect additional donor stem cells, or donor lymphocytes, for those recipients who relapse following cord blood stem cell transplant.

Transplantation biology

The rate of cord blood stem cell reconstitution and the kinetics of engraftment are slower when compared to bone marrow grafts (6,7,8). The factors which appear to be predictive in the outcome of cord blood stem cell transplants are total nucleated cell dose and HLA matching. Minor histocompatibility differences in allogeneic transplantation may contribute to graft rejection and graft-versus-leukaemia (9). The relatively slow kinetics of cord blood stem cell engraftment may be due to varying levels of adhesion molecule expression (10), homing characteristics (11,12), and the maturational stages of the cells. Nevertheless, it is also possible that donor lymphocytes contained in cord blood units are capable of inhibiting or even eliminating residual recipient immune cells capable of mounting a rejection episode (13). This observation may help to explain those cases in which engraftment has occurred in adults and in transplants of low graft CD34+ and low total nucleated cell doses.

The quantification of CD34+ myeloid progenitor cells and the relationship of this count to engraftment is not consistently predictive of outcome. There is a poor correlation between engraftment kinetics and CD34+ content of a given transplant possibly due to reduced surface epitope density of the CD34 antigen on cord blood stem cells (14). In addition CD34+ myeloid progenitor cells in cord blood have been shown in vitro to have a less mature phenotype compared to adult marrow and peripheral blood stem cells (15). This observation indicates that cord blood stem cells have a significantly increased proliferative potential when compared to adult marrow and peripheral blood stem cells (16).

Further evidence for this is provided by cobblestone area-forming cell (CAFC) assays which show the CD34+ compartment of cord blood to have a 3.6-10 fold increase in CAFC compared to adult bone marrow and peripheral blood stem cells (17). It is also interesting to note that the engraftment capacity of CD34+ cord blood myeloid progenitor cells in vivo using the nonobese diabetic/severe combined immunodeficiency (NOD/SCID) repopulation assay is significantly increased (17). Taking all of these unique properties into account starts to explain why cord blood stem cells engraft well despite low cellular content and also why late graft failure is rare (18).

Transplantation immunology

HLA mismatch between donor and recipient in allogeneic transplantation is an important factor in the development of acute and chronic GVHD. Nevertheless, transplantation of cord blood stem cells with HLA Class I and Class II mismatch results in a decreased incidence of acute GVHD when compared to recipients on unrelated adult donor stem cells (19,20,21). In this context it may be important that cord blood T lymphocytes are CD45RA+ and express low levels of activation markers indicating that they are the naïve Th0 phenotype (22). There a several possible explanations for the decreased incidence of GVHD following cord blood stem cell transplant including (23):

• Reduced donor lymphocyte numbers
• Donor T cell down regulation of recipient self antigen recognition and APC interaction
• Limited response of naïve donor T cells to recipient alloantigen
• Limitation of the cytokine/cellular cascade needed to amplify donor alloreactivity to recipient antigens
• Enhanced immunosuppression resulting from anti-thymocyte globulin and fludarabine used to achieve myeloablation in cord blood stem cell recipients

In vitro studies have shown that alloreactive T cells in cord blood grafts lack the full expression of immunomodulatory cytokines (22,24) and that the same cells in primary mixed lymphocyte culture show less cytotoxic effector function, less proliferation and greater activation induced cell death (AICD). In addition there is altered adhesion molecule expression on donor cord blood antigen-presenting cells (25). The presence of NK cells capable of early recovery and activation of the granzyme/perforin lytic pathway and Fas/Fas ligand (FasL) activity may also contribute to the low incidence of GVHD in cord blood stem cell transplantation (26). These same properties may also contribute to the delayed engraftment kinetics seen in cord blood stem cell recipients. Slow engraftment kinetics have been shown to correlate with increased risk of bacterial, fungal and viral infection following allogeneic transplantation. Despite this it has been reported that early post-transplantation infection in adults patients transplanted with HLA mismatched allogeneic cord blood stem cells is increased when compared to later infections which are the same as those patients receiving adult stem cell transplants (8). The high incidence of early post-transplant infection in cord blood stem cell recipients may reflect the neutropenia and lymphopenia seen following transplantation of relatively low numbers of nucleated cells and CD34+ cells. In addition adult recipients of cord blood stem cells tend to be in higher risk groups with extensive treatment prior to transplant (27).

There is clearly still much to understand about the immunology of cord blood stem cells in relation to GVHD and transplantation kinetics.

**Cord Blood Stem Cell Amplification**

Most cord blood transplants require 1-2x10^7 nucleated cells/kg in order to achieve safe engraftment kinetics and an average cord blood unit, after processing, contains 3-4x10^8 nucleated cells. This makes the ideal weight of a cord blood stem cell transplant patient in the range of 30-40 kg thus restricting the use of cord blood stem cell transplantation to children and small adults. In order to make cord blood stem cells available for transplant it is necessary to amplify the number of stem cells in the cord blood unit and extensive research is ongoing in the field.

Clonogenic cord blood progenitors, after the removal of more mature CD34- cells, have been expanded from 50- to 100-fold using cytokine cocktails and more primitive Delta cells also show a 10-20 fold increase in similar conditions (28). In addition Long term Culture Initiating Cells (LTCIC) in cord blood have been amplified 10-fold using low-dose cytokines and continuous perfusion technology (29). It must be noted however that none of the current amplification studies have assessed NOD/SCID repopulating cells and therefore the true extent of stem cell amplification in these studies is difficult to assess. Lewis *et al* (30) have subsequently shown that during *ex vivo* cytokine amplification of cord blood stem cells there was a 20-25-fold increase in Colony Forming Cells (CFC) whilst LTCIC only increased by 40% and NOD/SCID repopulating cells were maintained but showed no increase in number. This raises serious questions about the overall efficacy cytokine based of cord blood stem cell amplification in clinical practice. Further research may refine the technology to the point of true stem cell amplification.
An alternative approach is to amplify stem cells using bioreactor technology such as that pioneered by Cytomatrix. In this system stem cells are seeded onto a 3-dimensional, tantalum coated biomatrix and grow and amplify under the influence of naturally produced cytokines (31). This technology only achieves a 3-fold increase in CD34+ myeloid progenitor cells but may be an alternative source of amplified cord blood stem cells for transplantation in the future.

It is, of course, possible that simply increasing cord blood stem cell numbers available for transplant will not increase engraftment kinetics. Cord blood stem cells may require additional maturation and enhancements in the immune cells, antigen presenting cells and mesenchymal stromal cell components of the graft may also be required. The interaction of the cord blood stem cells and the rest of the cellular compartment may possibly enhance the overall engraftment kinetics of transplantation (32).

Cord Blood Stem Cells and Regenerative Medicine

Cord blood stem cells have enormous potential in the ever expanding field of regenerative medicine. A rare population of pluripotent CD45- cells have been identified in cord blood which grow in adherent culture and can be amplified up to 10^15 cells without loss in pluripotency (33). This CD45- population have been shown to be capable of differentiation into osteoblasts, chondroblasts, adipocytes, haematopoietic cells and neural cells. The neural cells derived from the CD45- population include astrocytes and neurons expressing neurofilament, sodium channel protein and neurotransmitter phenotypes. Transplantation of CD45- cells into the adult rat brain shows the presence of human Tau-positive cells for up to 3 months post-transplant and typical neuron morphology. Cord blood stem cells clearly have a potential in the future therapy of neurodegenerative disease and repair following traumatic injury to the central nervous system.

Another possible source of cells for transplant from cord blood can be obtained from the culture of CD34+ endothelial precursor cells (EPC) amplified to clinically useful numbers (34). These cells have been shown to proliferate in vivo, to form vascular structures and to improve experimentally induced myocardial infarction. The cells migrate to the infarcted myocardium where they engraft and participate in neoangiogenesis thus benefiting the remodelling process in post-infarction repair. Similar studies have shown that CD34+ cells obtained from cord blood can significantly improve ventricular function following myocardial infarction (35). These studies indicate a potential role for cord blood stem cells in the treatment of acute myocardial infarction and possibly cardiomyopathy.

Experimental studies have also indicated that cord blood stem cells have potential in the treatment of both type 1 and type 2 diabetes (36,37).

In summary cord blood stem cells represent a readily available source of stem cells for transplant for haematological malignancy and disease and repair of the bone marrow following high dose chemotherapy for cancer. Future applications are likely to be wide ranging and represent significant therapies in years to come.

References


Cord Blood: Opportunities and Challenges for the Reconstructive Surgeon

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A significant proportion of the activities of a Plastic and Reconstructive surgeon is directed towards the healing of wounds and the reconstruction of defects in the skin and soft tissue. In this Chapter we look at some of the opportunities and challenges presented by stem cells and in particular those derived from cord blood in the fields of chronic wound healing and skin repair and regeneration. It should be stated at the outset that our perspectives on new waves of biological evolution are influenced by experience, disappointments and frustrations: a fundamental problem being a consistent underestimation of the biological complexity of the human body not just in terms of the genetic source and cellular structure but also in terms of the extracellular matrix and composition of organs and tissues. When Rheinwald and Green described the serial cultivation of strains of human epidermal keratinocytes with the formation of keratinizing colonies from single cells over thirty years ago, naive claims were made by the laboratory scientists that the burns care of the future would be a simple matter of quick and easy cover after the excision of burn wounds. Unfortunately this is far from reality (1). The next decade brought the concept of tissue engineering.

When the term ‘tissue engineering’ was officially coined at a National Science Foundation Workshop in the USA in 1988, it was understood to mean ‘the application of principles and methods of engineering and life sciences toward fundamental understanding of structure-function relationships in normal and pathological mammalian tissues and the development of biological substitutes to restore, maintain or improve tissue function’. This concept has unfortunately led to some serious misconceptions that have resulted in the early promise of skin tissue engineering being slow to be realized in clinical practice. The misconception was that skin was a tissue, like cartilage, and would be relatively simple to address as a tissue engineering challenge. Skin however is NOT a tissue but an extremely complex organ that brings into conjunction cells from three different embryological origins, ectoderm, mesoderm and neural crest. It subserves multiple functions. The original futuristic claims of producing ‘off-the-shelf’ skin replacements have become far more restrained in their expectation and now tissue engineering skin products are being described as skin substitutes to aid healing and repair, temporary skin replacements and occasionally aids to regeneration.

Structure and Function of Skin
Skin is the largest immunologically competent organ in the body (1-2). It extends to over 1.6 square meters in the adult and weighs approximately 3000gm. The skin has two layers, epidermis and dermis (Fig 1). The epidermis is rich in cells and is of ectodermal origin. The cells are specialized in the formation of keratin and are called keratinocytes. The basal keratinocytes adhere to the basement membrane, which forms part of the zone between epidermis and dermis: the dermo-epidermal junction. Basal keratinocytes are unique in that they are capable of proliferating to form either new basal cells or terminally differentiating keratinocytes. The terminally differentiating keratinocyte moves up through several layers and undergoes certain changes: the cytoplasm becomes increasingly packed with keratin and the nucleus shrinks. The outermost layer—the stratum corneum—contains dead keratinocytes packed with keratin, forming a barrier between the living tissues and the external environment.
The area of the basement membrane far exceeds that of the surface of the stratum corneum, because multiple dermal papillae project from the surface of the dermis and only about 12% of the basal cells are proliferating at any one time. These papillae are formed by loose approximations of collagen bundles referred to as the papillary dermis, which lies on the relatively much thicker reticular dermis. Collagen bundles in the reticular dermis are thicker and more condensed.

Between the organized structure of elastin and collagen fibres is a thick, viscous fluid made up of glycosaminoglycans and hyaluronan. This arrangement of the dermis gives the skin its major biomechanical properties, allowing stretching and recoil and deformation without destruction.

The dermis contains a complex vascular arrangement of capillary and venous plexuses. Within the dermis are adnexal structures of ectodermal origin—hair follicles, sweat glands, and sebaceous glands—which are lined with keratinocytes. This arrangement becomes particularly important when considering the mechanisms of wound healing.

Multifunctional Langerhan’s cells found in the epidermis have an important role as antigen-presenting cells in cutaneous immune reactions. Skin colour is determined principally by the pigment melanin, produced by melanocytes. These cells package the melanin into melanosomes, which are then transferred to keratinocytes.

The skin is richly endowed with sensory nerve endings that enable the skin to play its vital role as a tactile interface between the body and the environment. Other sensory functions provide important stimuli for behavioural modification, e.g., the withdrawal reflex associated with pain or excessive temperature.

Healing: Regeneration and Repair
Healing in the skin takes place by two principle processes: regeneration and repair (Fig 2). Regeneration is the capacity of a tissue to renew itself so that the end result is indistinguishable from the preinjured tissue. Regeneration is a feature seen in superficial partial-thickness burns, in which the injury involves the loss of epidermis and basement membrane and the papillary dermis. There may be a highly exudative and painful wound.

The exudative phase persists for several days, and as it decreases the nature of the exudate changes. The viscosity and relative protein content increase, and eventually a fibrin layer seals the wound. In the meantime, the basal keratinocytes at the margin of the wound begin to undergo mitosis. In the normal resting state only approximately 12% of basal keratinocytes are proliferating at any one time, giving the skin a tremendous reserve capacity. Re-epithelialization begins not just at
the wound margin, but also from the appendageal structures. The rate of keratinocyte proliferation and migration is extremely high, and when the exposed dermis is completely covered with a new keratinocyte layer, contact inhibition stops migration and redirects the cells to stratification. A superficial partial-thickness burn or abrasion will heal with a stratified squamous epithelium in a matter of days. Disturbance in normal pigment expression can occur, even with no scarring. In people with darker pigmentation, areas of absent pigment can have major social and psychological sequelae.

As the wound becomes deeper, the nature of the healing changes. In a burn, damage to the dermis involves irreversible denaturation of the collagen. The inert collagen has to be removed for re-epithelialization to take place. Removal involves an autolytic process with enzymatic degradation and phagocytosis, augmented by an inflammatory response (Fig 3). Healing takes longer. As the depth of injury increases through the thickness of the dermis, the phenomenon of inflammation plays an even more important role in the healing process. Typically, inflammation initiates a cascade of events, with polymorphonuclear leukocytes being attracted to the wound site. Their principal role is proteolysis and phagocytosis of debris. The leukocytes release cytokines which cause macrophage activation. The activated macrophages enter the wound site to undertake a more detailed assessment of the damage and through further cytokine signaling, they recruit fibroblasts to begin the process of replacing the damaged collagen. Fibroblasts involved in wound healing have the capacity to produce abundant amounts of collagen, but they have lost the capacity to place and organize it in a highly structured way. The end result of dermal repair is the deposition of disorganized collagen, which is physically apparent as scar tissue. Scarring represents a very complex biological phenomenon. Hypertrophic and keloid scars represent clinical descriptions of a heterogeneous group of disorders with various etiologies and pathological mechanisms that result in the production of a disorganized connective tissue (4). The duration of the phase of wound closure (i.e., re-epithelialization) and the incidence of adverse scarring increase as the depth of injury increases.

**Fetal Wound Healing**

Early in gestation, fetal wounds are capable of healing without scarring. That is to say that there is regeneration of tissue. The nature and mechanism of this scarless repair has been intensively investigated as the achievement of regeneration in the post-natal wound would have overwhelming benefits for mankind. The cellular mediators of fetal skin repair have been studied including platelets, inflammatory mediators and fibroblasts. The extracellular matrix has also been extensively studied as well as the effects and influence of cytokines such as TGF-β, PDGF, fibroblast growth factors and vascular endothelial growth factor. Despite intensive research it appears that the ability to heal scarlessly is intrinsic to fetal skin (5). Nevertheless, this is another area where the potential control and interaction of stem cells is and will be the focus of considerable research.
The Need for Skin Substitutes

Skin substitutes are needed to augment the healing process of chronic wounds such as diabetic and vascular ulcers (6). The abnormalities associated with these ulcers include systemic factors (advanced age, malnutrition, diabetes and renal disease), local factors (prolonged infection, ischemia) and decreased synthesis of collagen, increased levels of proteinases and defective macrophage function (7).

Skin substitutes are also needed for wounds that arise from extensive tissue loss or damage. These may result from trauma, in particular burns, and in pathological conditions such as epidermolysis bullosa and acute exfoliative skin conditions. Such wounds may need either temporary or permanent closure with substitutes.

The Ideal Skin Substitute should be as following (8-10):

1. Protect the wound, and maintain a moist healing environment and control protein and electrolyte loss.
2. Prevent local infection and provide an environment for accelerated wound healing.
3. Reduce pain and allow early mobilization.
4. Be easy to handle and cost-effective.
5. Must be safe in terms of virus transmission and not provoke a strong immunological reaction.
6. Should be readily available.

Strategy of Skin Tissue Engineering

Strategies used to construct skin substitutes in tissue engineering are generally considered to be either: ex vivo tissue manufacturing with guided generation or in vivo regeneration (11). These are shown in Table 1. The strategy of ex vivo tissue manufacturing is the technique initially most commonly associated with tissue engineering. In this approach, fibroblasts and/or keratinocyte are seeded into dermal matrix or scaffold and co-cultured in a bioreactor or specialized culture system with some growth factors. The matrix provides a scaffold combining with the bioreactor providing cellular nutrients, allowing the cells to proliferate and differentiate in the ex vivo environment. When the procedure is completed, the skin substitutes is implanted into the wound and further matures and integrates into the recipient tissues. Such available products include: Epicel™-cultured epidermal sheet (12), Dermagraft® (13), Apligraf® (14) and cultured skin substitute (15).

The other strategy is to guide the ‘bioengineering’ of skin in situ. Such a strategy needs an understanding of the cellular and molecular interactions in tissue healing and development. Examples
are Alloderm® and Integra®, which can act as 'dermal generation templates' in vivo to direct the formation of a new autocollagenous 'dermal' matrix (16-18).

The Keratinocyte Layer

A major problem with the keratinocyte layer is its extreme antigenicity, mediated principally through Langerhans cells. This has presented a major obstacle in the restoration of epidermal cover by tissue engineering. The major contributions have been to develop materials to carry cells, cultured in the laboratory, onto the wound.

In 1975, when Rheinwald and Green first described the technique of producing keratinocyte cultures in vitro, it appeared that a new era of wounds management was about to commence (19). Indeed it has, but the expectation of rapid wound closure with laboratory-produced cultured epithelial cell autografts (CEAs) has not been fulfilled in clinical practice. The process of preparation is expensive. Although it takes only a few weeks to create enough CEAs from a few square centimeters of original biopsy to cover the entire body, the actual take and survival of the CEAs on the body has been a disappointment. Keratinocyte delivery vehicles have been developed to facilitate the process and improve the rate of successful engraftment, but this has further increased the costs of this strategy while not significantly increasing the success (20).

There has been a tremendous amount of research and development in keratinocyte preparation and application, and there has been a parallel development in the area of dermal replacement and/or regeneration. One major problem that has faced workers in this field is how to combine the two layers of the skin. The dermo-epidermal junction (DEJ) is extremely complex. It is a combined unit with structural components produced by both keratinocytes and by dermal fibroblasts. In nature, a new DEJ is formed when proliferating keratinocytes migrate across the dermal collagen. This occurs in all superficial wounds and in healing donor sites. There is no problem in the function of a DEJ in these situations, and this focused attention on the role of the undifferentiated, proliferating keratinocyte cell suspensions in burn wound coverage. Fiona Wood, a pioneer in this field, has used cell suspensions of keratinocytes to treat burns in an increasing number of clinical applications, including dermal collagen remodeling, pigment expression, and keratin expression (21). Thus, the interaction between the products of the keratinocytes and the dermal fibroblasts becomes as important as the speedy regeneration of the lost epidermis.

The Challenge

The 21st Century tissue engineer faces the challenges of both possibility and practicality. Complex and costly products will not find commercial applications. In the meantime the biological complexity of
the skin has been appreciated and it is no longer viewed as a bilaminar tissue but a multidimensional organ. The tissue engineer must ask how much of this complex biological tissue is going to be constructed. Figure 4 shows the ‘biological’ as opposed to the ‘engineering’ concepts of the skin structure. The skin began in the embryonic stage as a conjunction of cells which integrated their specialized functions. The key cell of the epidermis is the keratinocyte which produces keratin. It is the outer layer of keratin filled, dead cells, the stratum corneum that is responsible for the principle protective functions of the skin. The mesodermal fibroblasts are principally involved in the formation and maintenance of the dermis. The problem for the tissue engineer is that the time scale for cellular matrix production is too slow in clinical application. A preformed matrix is required either as a permanent or temporary scaffold. The problem with the permanent scaffold is biocompatibility and the problem with the temporary scaffold is stability.

For the permanent replacement of lost skin it will be necessary to use a dermal analogue which is slowly replaced by an autologous dermis. Such a product might be formed by an immune modulated allogeneic or xenogenic dermis modified with mesenchymal stem cells, either of marrow or adipocyte origin. This layer would need to be seeded with a stem cell enriched keratinocyte formulation possibly including melanocytes. The attachment is critical and will require a medium such as hyaluronic acid which can support the cells whilst promoting proliferation and attachment. These ex vivo derived components would be combined for in vivo culture. Such a process can be simply conveyed diagrammatically (Fig 5) but the practicality of such a construct presents considerable challenges. The history of the evolution of skin tissue engineering does suggest however that for permanent skin replacement the body’s own ‘tissue engineering’ capacity must be harnessed using stem cell technology with or without synthesized matrix support. For tissue engineered skin substitutes to provide temporary wound cover materials science and technology already shows promise but the challenge is to reduce costs. Again for products which are aimed at modulating wound healing, living cells can be genetically engineered to restore molecular balance to chronic wounds. Again knowledge and technology are available but the challenge is to develop products that are safe, effective, easy to use but also affordable.

**Stem Cells**

From the foregoing it is apparent that the skin is indeed a complex structure incorporating a fusion of multiple cell types, integrated within a three dimensional matrix containing both fibrillar and non-fibrillar elements. To synthesize such a complex structure by identifying the component parts and to put them together is neither practical nor realistic. It must be observed, however that this integrative strategy has been the major one used in skin tissue engineering during its less productive phase (22). The great attraction of stem cells in the construction of a complex tissue or organ is that the component parts can be simplified at the initial stage and a significantly greater proportion of the neogenerative process can be driven by the intrinsic bioengineering capacity of the cells and tissues. It is important to realize that cells by themselves cannot generate organs and the matrix provides a critical element in defining appropriate differentiation and three-dimensional organization. Stem cells
are going to play an increasingly important role in tissue engineering and they will be derived from a range of sources.

Stem cells can be variously classified. One way is to identify the source and thus we can regard stem cells to be embryonic, foetal or adult in nature. An alternative perspective is to look at the future, the potential and to describe stem cells as being totipotent, pluripotent and multipotent.

The totipotent cells contain all the complete genetic information needed to manufacture all the cells of the body as well as the placenta. These cells are present immediately after fertilization of the egg and for three to four divisions thereafter. As the cells become more specialized they are described as pluripotent. The cells are extremely adaptable and can develop into any cell type with the exception of the placenta. Further division of the pluripotent cells will give rise to multipotent cells. These are far more specialized and can only generate a limited number of cell types (23).

The true stem cell must satisfy certain criteria: it must be clonogenic i.e. capable of unlimited self-renewal by symmetric division; it must also be able to divide asymmetrically, with one daughter cell resembling the mother (to perpetuate the clone) but the other capable of giving rise to multiple types of differentiated cells which indeed represent derivatives of all three primitive embryonic germ layers.

It is the concept of 'plasticity' which makes the stem cell so attractive to the tissue engineer and so one critical aspect of stem cells will be the variable expression of plasticity related to source. In Table 2 the potential plasticity of some adult stem cells is detailed which does indicate the wide range of cells that have thus far been generated from bone marrow cells (24). It is evident, however, that this plasticity is limited in adult stem cells certainly compared to stem cells derived from the inner cell mass of the early embryonic blastocyst (ES cells) which can both proliferate indefinitely but also give rise to virtually any type of cell (25).

**Adult Stem Cells**

Adult stem cells have been incorporated into tissue engineered constructs used by reconstructive surgeons. A recent review has focused on the four components in developing tissue substitutes: gene therapy, growth factors and pharmalogical preparations, scaffolds and cells. It is this last component, the cells, where increasing attention is focusing on stem cells particularly in true tissue as opposed to organ-engineering. Bone, cartilage, tendon and muscle have all been developed to some degree of success from adult stem cells. The preferred source of adult stem cells however remains uncertain and the functional plasticity of adult stem cells exists more as of scientific experimentation than clinical application (26-27).

**Cord Blood**

In view of the many ethical and biological issues involved it will be many years before embryonic stem cells reach the clinic. Research in this field has not been helped by the highly publicized claims of scientific fraud by some high profile researchers (28). Nevertheless the attraction of supplies of
pluripotent stem cells remains as a prized resource by scientists and clinicians. The annual global 100 million human birth rate underlines the possibility that umbilical cord blood (UCB) represents the world’s largest untouched stem cell resource. The particular advantages of stem cells from this source include ethical acceptability, a naive immune status and relatively unshortened telomere length. Claims are already being made that stem cells with embryonic characteristics can be produced from human umbilical cord blood (29). Cord blood is being used for an increasing number of clinical and experimental applications which have highlighted the reduced incidence of graft versus host reaction when the haemopoietic fraction is used as an allogenic transplant (30).

As more uses are found for umbilical cord blood (UCB), considerable attention has been addressed to expanding the number of stem cells at differing stages of maturity. Expansion factor of 10 to more than 1000 have been claimed and one aspect of considerable significance is that undifferentiated stem cells can be expanded. Cell process engineering for expansion does rely in three-dimensional matrices in bioreactors (31).

Plasticity of Cord Blood
Cord blood and cells derived from the umbilical cord have been used in a variety of tissue engineering projects. Living patches of tissue fabricated from synthetic polymers (PGA/P4HB) seeded with fibroblasts harvested from umbilical cord tissue and endothelial progenitor cells have been cultured in a perfusion bioreactor and have the potential of being used in congenital cardiac conditions (32). Attempts to engineer microvessels using similar endothelial progenitor cells and polyglycolic acid-poly-L-lactic acid (PGA-PLLA) scaffolds demonstrated a lack of vessel formation. However when the same cells were co-cultured with human smooth muscle cells, microvessel formation was observed on the porous (PGA-PLLA) scaffolds (33). Skeletal myogenic differentiation has been observed in mesenchymal cells isolated from UCB (34).

The response and interaction of stem cells and target cell differentiation is a focus of particular interest to the Plastic Surgeon. The review by Heng et al discusses strategies for directing keratinocyte stem cell lineage in vitro using selective purification and proliferation (35). A three step process is described with the first step being to induce commitment of the (non-epidermal) stem cell into keratinocyte progenitors. These progenitors have to then be selected and purified and finally this purified population of committed keratinocyte progenitors has to be expanded by proliferation and allowed to differentiate.

Various strategies are discussed for committing stem cells to the keratinocyte lineage including induction with exogenous cytokines, growth factors, chemicals and extracellular matrix. It is becoming obvious that stem cell biology is potentially very complex if such strategies are to be adopted to direct stem cell differentiation and maintain it.

It is evident, however, that there are going to be considerable challenges in the development of reliable protocols that can confidently preclude risks of teratoma formation and other, as yet
unforeseen complications. This will certainly have an impact on the introduction of widespread clinical applications of mesenchymal stem cells in tissue engineering.

Wound Healing Modulation

One area of reconstructive practice that may be of more immediate clinical application is the use of stem cells to promote and modulate the healing of wounds. In this situation the cells will be placed into a pathological environment for example a chronic non-healing wound. They will undertake an assessment of the physiological deficiencies in terms of matrix composition and cytokine milieu and correct these by producing the appropriate wound healing modulators. In a sense this means that the cells are acting as an intelligent, interperative biofeedback control mechanisms that can autoregulate biological systems. Bone marrow, peripheral blood and umbilical cord blood have all been used in chronic wounds to modulate the healing response. Whilst the early experience is limited, the prospects are promising and some of the concerns about incorporating stem cell-derived tissue, into the body are unwarranted (36).

Of particular interest is the effect of topical applications of bone marrow derived cells on chronic wounds. Although the reports are few the consistent theme is that a chronic wound changes its nature to become an acute wound that heals or becomes healthy and can be closed with a skin graft (37-39). Laboratory studies looking at cutaneous healing in a chimeric mouse model indicate that when marrow is transplanted it can contribute to the reconstitution of the dermal fibroblast population in the wound although local cutaneous cells reconstitute the epidermis (40). An in vitro study indicated that collagen synthesis and levels of bFGF and VEGF were much higher in bone marrow stromal cells than those in dermal fibroblasts. This suggest the potential of topically applied bone marrow cells to accelerate wound healing (41). A further study has looked at the effects of a bone marrow-impregnated collagen matrix and found a significantly increased angiogenic effect in an experimental mouse model. This same group applied an autogenous bone marrow-impregnated collagen matrix to a patient with a chronic leg ulcer and observed a dramatic healing response (42).

In our own clinical experience we have applied autologous bone marrow to a chronic unhealed burn wound, a donor site that had repeatedly failed to heal and a chronic wound at the extremity of latissimus dorsi free muscle flap where the graft was being traumatized by footwear. The burn wound changed from being chronic and non-healing to re-epithelializing and was closed with a graft. The donor site that had failed to heal and also had repeatedly failed to take a graft, healed with no grafting necessary. The chronic heal wound became more vascular and was definitively closed with a skin graft. Another recent report describes the use of allogenic bone marrow mesenchymal stem cells for the treatment of a patient with deep skin burns (43).

It is in these cases that we see the great potential for topical application of cord blood as a biological wound healing modulator. It is of interest to note that amniotic membranes have been used in the past as biological dressings for wounds but concerns about risks of disease transmission have severely limited this practice in many parts of the world. Similarly the question of potential risk of
disease transmission when using cord blood may also be raised. However there are already well defined screening processes to reduce and/or eliminate such risks as applied in routine blood banking. Another consideration is that this use of cord blood is temporary and the relative lack of immunogenicity will limit adverse effects.

As the understanding of the range and nature of the stem cell composition in cord blood becomes more clear, it may be possible to apply more selective fractions onto wounds both chronic and acute to modulate the biological healing mechanisms. It would be a mistake to underestimate the complexity of stem cell biology and the clinical applications. Nevertheless there is hope that the resource provided by umbilical cord blood will make a major contribution in the provision of cost-effective care of both acute and chronic wounds throughout the world.
References


(28) Jones N & Cyranoski D. Investigation says Hwang lied. News (doi:10.1038/news051219-17)


Legends

Figure 1  A diagrammatic section of skin.
Figure 2  Wound healing – the pathways.
Figure 3  Wound healing – repair.
Figure 4  Biological concepts of Skin Tissue Engineering.
Figure 5  The conceptual process of construction.

Table 1  Comparison of ex vivo and in vivo strategies.
Table 2  Potential plasticity of adult stem cells [adapted from Rosenthal N (24)].
Figure 1
Figure 2

TISSUE INJURY

WOUND
‘A discontinuity in tissue integrity’
(functional and/or structural)

HEALING
‘Restoring tissue integrity’

REPAIR
SCAR

REGENERATION
NO SCAR
INJURY

Blood Clotting
Platelet aggregation & activation

Leukocyte migration and Macrophage activation

Soluble Mediators

Fibroblasts → Extracellular matrix
Endothelial cells → Angiogenesis
Keratinocytes → Re-epithelialization

Collagen Lysis / Collagen Synthesis Contraction
Maturation

Remodelling

SCAR

Figure 3
- Clean Dermal Analogue
- Modify + stem cells
- Stem cell rich keratinocytes (+/- melanocytes)
- Culture in vivo
**Ex Vivo**

- Laboratory based tissue manufacturing
- Complex and tissue consuming technical processes
- No intrinsic blood supply
- One stage procedure but usually temporary

**In Vivo**

- Guided generation or regeneration of tissue
- Need for significant understanding of biological processes and gene control
- Develops blood supply in situ
- Multiple stage procedure but can be permanent

<table>
<thead>
<tr>
<th>Location of Stem Cell</th>
<th>Type of Cells Generated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Neurons, oligodendrites, skeletal muscle, blood cells</td>
</tr>
<tr>
<td>Bone marrow</td>
<td>Endothelial cells, blood cells, cartilage, bone, adipocytes, cardiac muscle, skeletal muscle, neuronal cells, dermal fibroblasts, oval cells, gastrointestinal tract cells, thymus, pulmonary epithelial cells.</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>Skeletal muscle, bone, cartilage, fat, smooth muscle</td>
</tr>
<tr>
<td>Myocardium</td>
<td>Myocytes, endothelial cells</td>
</tr>
<tr>
<td>Skin</td>
<td>Keratinocytes</td>
</tr>
<tr>
<td>Liver</td>
<td>Liver cells</td>
</tr>
<tr>
<td>Tests and ovaries</td>
<td>Gonads</td>
</tr>
<tr>
<td>Pancreatic ducts</td>
<td>Islet cells</td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>Fat, muscle, cartilage, bone</td>
</tr>
</tbody>
</table>

**Table 1**

**Table 2**
Possibilities of using cord blood for improving the biocompatibility of implants.
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Abstract
Cord blood is a rich source of stem cells, growth factors and immune suppressing cytokines. We have reviewed its potential for enhancing material integration in the case of implants. We have reviewed the problems with the current degenerative therapy, future outlook and also the potential of using cord blood to mimic the biological way of delivering active molecules, immune suppression and tissue regeneration.

Introduction
Degenerative disease affecting different organs like kidney, liver, heart etc., often end up in loss of tissue or organ function. The current therapy of most of these diseases is by using medical devices or using tissue, from different sources for the partial recovery of their functions. These treatment methodologies are often assisted with drug therapy to improve the patency of the implants. The continuous monitoring and assisting drug therapy often seems to be costly and cumbersome. The synthetic medical devices reduce this problem to certain extent because of its improved durability as compared to its tissue counterparts. However substitution of synthetic implants seems to be impossible at certain regions where correction is needed than replacement of the organs. For eg:- urinary bladder, and heart tissue etc.
The major problem associated with the medical devices inside the body is performance failure due to biological reactions, regulated by the adsorbed proteins and the pathological cells on the material surface. Usually the surface of the material is being modified to make the material biocompatible. Introducing specific surface groups, immobilising proteins with certain conformations, or by immobilising certain cell lines, often does this. Strategies have also been adopted to modify the material/biology interphase. Basically this type of cell-mediated therapy is being done to improve the device integration by augmenting the tissue regeneration. For e.g:- endothelialisation of the vascular grafts to improve the blood compatibility(1); Utilizing platelet rich plasma for improving the tissue regeneration in periodontitis (2) etc.
However the lessons from the organ morphogenesis, and wound healing suggests that specific chemokines are being sequentially released during the tissue regeneration. The tissue regeneration and wound healing are the important events after any tissue damage. Whether it is due to trauma or implanting a medical device. Here the difference is that when a medical implant
is placed in the surgical wound the implant surface along with the adjuvant medication seems to regulate the normal wound healing process. Surface modification of the devices for improved biocompatibility by varying techniques both by mimicking the biology as well as by understanding the biological processes is in extensive research today. The cells are migrated from the neighboring environment for the wound healing. Alternative methods by making biofriendly surfaces with, endothelialisation (1) and immobilization of the ECM proteins (3), looks promising. However immune rejection cannot be neglected in this case. Stem cells are an alternative choice for this problem, as Human Leukocyte Antigen (HLA) matched stem cells are less prone to immune rejection (4).

Cord blood is a rich source of growth factors, immunosuppressant chemokines and stem cells. Here we have reviewed the possibilities of using cord blood as a "cocktail" of stem cells, growth factors and immunosuppressant cytokines for the integration of implant to the biological environment.

**Degenerative diseases and the current treatment methodology**

Many of the degenerative diseases affecting various vital organs leads to functional loss of that tissue or organ. In the case of organs that are performing mechanical functions like heart, blood vessels, bone etc. artificial implants of synthetic origin is being used. However in the case of organs that are performing synthesis of hormones e.g. pancreas or partial loss of organ function like heart valve the synthetic implants seems inferior to allogenic or xenogenic tissue implants (5). However tissue engineering looks to be an alternative approach in all these cases. Table-1 gives the current strategy and future promises in the treatment of degenerative diseases affecting vital organs. The current tissue engineering strategies are either partial functional restoration as in the case of artificial pancreas or surface modification using endothelialisation. Immune reaction to the xenogenic tissue seems to be an important problem in these cases. Despite these allogenic or immune protected In vitro cultured tissue grafts, attempts have also been made for tissue regeneration at the site of injury. This has been achieved with chimeric-morphogenesis with the help of porous scaffolds and bioactive molecules (6). Here the number of allogenic cells at the wound site is important for tissue regeneration, wound healing and integration of the material into the body. This is to avoid the overgrowth of the other cells that may get migrated to the site.

**Table- 1 Different degenerative diseases and treatment methodologies (current and future perspectives)**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Disease</th>
<th>Current Treatment</th>
<th>Problems to be resolved</th>
<th>Future trends based on biomimicry</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Aneurysmal</td>
<td>Surgical intervention</td>
<td>Pseudointimal</td>
<td>Tissue engineering</td>
<td>(7,8)</td>
</tr>
<tr>
<td>Disease</td>
<td>Cause</td>
<td>Treatment/Procedure</td>
<td>Complications/Special considerations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------</td>
<td>---------------------</td>
<td>---------------------------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vessel disease. Abdominal aortic aneurysm. Popliteal Aneurysm. Advanced atherosclerosis. Traumatic Injury</td>
<td>and replacing the vascular grafts of both the biological and synthetic origin (Eg:- Cephanous vein and PTFE Grafts)</td>
<td>hyperplasia Early thrombosis.</td>
<td>of vascular grafts and Endothelialization</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart Valves Symptomatic valvar heart disease</td>
<td>Surgical intervention and Mechanical devices and Prosthesis (Both allogenic and synthetic vascular grafts)</td>
<td>Lesser half life for the tissue based vascular grafts as compared to the synthetic one.</td>
<td>Tissue engineering of vascular tissue and grafts. (9,10)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver Hepatic encephalopathy</td>
<td>Total liver transplantation</td>
<td>Scarcity of the liver source.</td>
<td>Liver assist device with liver tissue regeneration. (11,12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney Chronic renal failure</td>
<td>Kidney transplantation, Hemodialysis, Chronic ambulatory peritoneal dialysis.</td>
<td>---</td>
<td>Tissue regeneration of the kidney tissues. (13,14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreas Diabetes mellitus</td>
<td>Drug therapy to increase the synthesis or utilisation of the insulin or direct administration of insulin.</td>
<td>&quot;Tolerance&quot; on prolonged drug therapy leads to the associated events in other organs like kidney or liver.</td>
<td>Tissue regeneration of the islets of langehans of the pancreas. (15,16)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Stem cells - cells with high plasticity**

Stem cells being totipotent and compatible with all the cell lineages could be used as an alternative strategy for this purpose. They have the capacity to self renew and give rise to differentiated progeny. These stem cells are present in virtually all parts of the human body such
as liver, muscle, brain, blood, bone and even in teeth. The introductory chapters have given a detailed introduction about the plasticity of stem cell plasticity, here we are discussing about the potentialities relevant to in vivo tissue engineering required for the surface modification of biomaterials, with the help of cord blood.

The haematopoietic stem cells and the adult stem cells present in the stem cell niches of various tissue help in renewal and regeneration of the tissue under damage (17). The specific signal molecules called growth and differentiation factors stimulate the differentiation of these stem cells, first to transit amplifying cells and then to terminally differentiated cell lineages. The stem cells can be switched to differentiate into various cell lineages and also could be transdifferentiated or dedifferentiate back, in an altered environment (18). The plasticity to cross the different cell lineage boundaries invoked significant scientific attention, and invited research in understanding the molecular mechanism governing differentiation. This would possibly have therapeutic impact in future in the area of degenerative diseases. Cord blood is a rich source of stem cells is being extensively explored for bone marrow transplantation (BMN) (19). Cord blood stem cells (CBSC), different from other stem cell sources like embryonic (ESC) and bone marrow stem cell (BMSC) have demonstrated rapid colony inducing ability (20), and less immune reaction. However the stem cells are to be differentiated to the corresponding cell lineages prior to its application. The teratoma formation due to undifferentiated stem cells has been identified (21).

Various growth and differentiation factors have been proposed to improve the proliferation as well as the differentiation of these stem cells.

**Inductive factors in cell proliferation and differentiation - biomimicry in drug delivery**

Prof. Hans spenman, one of the pioneers in developmental biology, who won the nobel prize in 1935, demonstrated that the inductive signals generated in embryonic tissue can regulate the differentiation in neighbouring tissues. (22, 23). This invited a lot of scientific interest in understanding the inductive stimuli and their role in governing the molecular mechanism of the morphogenesis. The present understanding is that the inductive chemical stimuli can be in the soluble form like growth factor, cytokines etc. or in the insoluble form like the peptide sequences of the extracellular matrix proteins or adhesion proteins like fibronectin or vitronectin. Both kinds of the inductive stimuli seem to do transmembrane signaling by binding to specific receptors. These receptors are G protein coupled will act on to different types of effector enzymes like adenylyl cyclase, phosphodiesterase and phospholipase C and through the secondary messengers like cAMP acts on to specific phosphatases and protein kinases. This will initiate the different cytoskeletal and nuclear cell activation processes leading to the different cellular effects including differentiation and proliferation (24).

There are evidences that the collaboration of the inductive stimuli (growth factor and matrix mediated) in cell proliferation (25).
In a wound the thrombus formed acts as a scaffold for the cells to migrate and grow, in addition to stopping the loss of blood. Here the bioactive molecules are immediately delivered to the site by the platelets and from the damaged cells (26). After the initial cleansing of the wound site by the neutrophils macrophages are invited to the site to remove the particulate matter and synthesis of growth factors. They further invite fibroblasts to synthesize the collagen, which form the basic structural unit of the extra cellular matrix. All these cell-cell communications happens sequentially regulated by specific inductive signals (cytokines and growth factors) in normal wound healing and will be altered under infection or in the presence of a xenobiotic (27). However the combination as well as exact mechanism of action of these inductive signals is far from understood.

To simulate the biological mode of delivering these soluble active molecules various techniques have been adopted at different fields for tissue culture. Co-culturing of two different cell lines has been proposed and is being successfully utilized in tissue engineering (28). Otherwise this is the basic principle of using serum for tissue culture (29). Combination of specialized differentiation factors and serum has been proposed for tissue engineering as well as stem cell differentiation (30). This is an alternative successful approach for co culture systems. Development of cocktails for specific tissue regeneration using these various bioactive molecules seems to have large market potential and invites a lot of research. For eg: - Haematopoietic growth factors such as human recombinant erythropoietin, GM-CSF and G-CSF are commonly used in allogenic and autologous stem cell transplantation (31). Cord blood is a rich source of this bioactive molecules and stem cells (32). Apart from that under in vivo conditions they form the interface between the mother and the foetus, which also appears that it can possibly regulate the growth of neighboring cell lineages without inducing an immune reaction. However its potential for generating a bioactive interface for implant is not being explored. Table-3 gives some of the examples for growth and differentiation factors using for stem cell differentiation.

Table-3 Family of growth and differentiation factors tried for stem cell differentiation

<table>
<thead>
<tr>
<th>Growth factor</th>
<th>Tissue</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukocyte Inhibitory factor (LIF)</td>
<td>Suppresses differentiation by activating transcription of pluripotent gene Oct-4.</td>
<td>33a, 33b</td>
</tr>
<tr>
<td>Transforming growth factor α (TGF α)</td>
<td>Growth and proliferation</td>
<td>34, 35</td>
</tr>
<tr>
<td>Bone morphogenic protein (BMP)</td>
<td>BMP's is Used for chondrogenic differentiation, and cartilage formation</td>
<td>36, 37</td>
</tr>
<tr>
<td>Fibroblast growth factor</td>
<td>Growth and proliferation</td>
<td>38,39</td>
</tr>
</tbody>
</table>
Apart from this soluble inductive signals the insoluble signals (i.e. the peptide sequences from the precipitated protein of the extracellular matrix) also have to be found to generate the transmembrane signal for the cell growth and differentiation. Table-4 give some of the evidences for peptide sequences which regulate cell attachment and differentiation.

<table>
<thead>
<tr>
<th>Peptide Sequences</th>
<th>Parent protein</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>RGD</td>
<td>Fibronectin, collagen, fibrinogen, laminin, vitronectin, Von Willebrands factor, entactin, tenascin, thrombospondin</td>
<td>48, 49</td>
</tr>
<tr>
<td>YIGSR</td>
<td>Laminin</td>
<td>50, 51</td>
</tr>
<tr>
<td>IKVAV</td>
<td>Laminin</td>
<td>52, 53</td>
</tr>
<tr>
<td>LRE</td>
<td>Laminin</td>
<td>54, 55</td>
</tr>
<tr>
<td>REDV</td>
<td>Fibronectin</td>
<td>56, 57</td>
</tr>
<tr>
<td>DGEA</td>
<td>Collagen</td>
<td>58, 59</td>
</tr>
<tr>
<td>GXG</td>
<td>Thrombospondin</td>
<td>60, 61</td>
</tr>
<tr>
<td>VGVAPG</td>
<td>Elastin</td>
<td>62, 63</td>
</tr>
</tbody>
</table>

These soluble and the insoluble cues direct the cell migration and proliferation at the wound site. Evidences also suggest that there is signal correlation inside the cell after receptor activation (64a,b). As Prof. Vacanty foresees one of the next breakthrough research will be circumventing the immune response of this tissue engineered materials (65), here is an example of tissue integration for specified duration regulated by humoral response. Attempts in this direction may help in reducing the immune rejection of implant material also.

**Immune reaction to the implant - A lesson from foetal survival**

The fertilized egg (embryos) seeks nutrients from mother by forming contact through the placenta. The reason for tolerance of the embryos by the maternal immune system despite the presence of paternal MHC histocompatibility antigens has invited a lot of research. The local immune response at the site of contact is suppressed by the anti immune cytokines produced locally at the site of contact.
There is also clear evidence that the maternal immune system during pregnancy can enhance or inhibit the development of foeto-placental unit. Recent data supports that some cytokines produced by both T cells and non-T cells (IL-3, GM-CSF, TGF-β, IL-4, IL-10) favour foetal survival and growth. In contrast other cytokines such as IFN-α, TNF-α, and TNF-α, can rather compromise pregnancy. They have classified the human lymphocyte CD4+ T helper cells into two classes (Thelper1 and Thelper2) based upon these cytokine secretion profiles. The T helper 1 (Th1) cells produces IFN-α and TNF-α, while the second type, T helper 2 (Th2) cells produces IL-4 and IL-5 and a third type (Th0) is also observed, that produce both Th1 and Th2 cytokines. They concluded that the cytokine network maintaining the foetal survival mainly belong to Th2 pathway, whereas the failure of pregnancy is associated with the dominance by Th1-type cytokines. In vitro studies suggests that progesterone enhances the preferential development of Th2-like cells and enhances transient IL-4 production (66). While relaxin another corpus luteum derived hormone mainly promote development of Th1 like cells (67). Further during the development of the umbilical cord this principle is maintained and the cord blood contains more Th2 cytokines rather than the proimmune cytokines. At situations like urinary infection and associated abortion of the foetus the shift in paradigm of increased production of Th1 cytokines have been observed. This clearly demonstrates the endocrine immune relationship in maintaining the pregnancy.

Here is an equivalent situation when an implant, whether tissue or artificial implant, is placed in a wound (Implant site). When the body identifies the implant as a xenobiotic it first try to destroy the implant, if not possible isolate with the help of a fibrous capsule. If the inflammatory reaction persists due to variety of reasons like leachables, abration of the implant, infection at the implant site etc., lead to the immune response against the implant. This can be Type-I to IV depending upon the solubility, size, and surface chemistry of the xenobiotic formed from the implant. Most of the materials using for developing the implants are tested for each class of immune reaction preclinically, and qualifies for the implantation (68). However taking care of the difference in pathogenesis of various microorganisms, between the animals (using for preclinical studies) and human, certain clinical evaluation protocols are also to be followed to avoid any hyper sensitivity cascades during implantation (69). Both the humoral immunity due to the formation of antibodies against the soluble leachables and cell-mediated immune reactions against the particulate material due affect the biomaterials. In that the cell-mediated immunity due to the surface identification of the biomaterials invites chronic inflammatory responses which will complementarily activate the humoral immune system and further decides the fate of implant. The nature of the lymphocytes at the implant site and their cytokine responses regulate the cell-cell signaling during chronic inflammatory response. Here also the Th1 cell response is higher
lead to excessive secretion of the IL-1, IFN-å, TNF-â, and TNF-á at the implant site (70, 71). These lymphokines are proinflammatory in nature, as said earlier. The comparison between the fetus and the implant illustrate, the implant exposes to a big area, and so more amount of proinflammatory agents are released, and with time the environment at the implant site decides, the paradigm shifts towards tissue integration or immune rejection. The lymphocytes at the implant site through different cytokine profiles regulate the “paradigm shift”, which is similar to the maintenance of foeto-maternal system. Different from the fetus, in the case of implant, if it is not identified as a xenobiotic they tend to integrate with the tissue, otherwise it will be isolated with a fibrous capsule. In this case the long-term chronic inflammatory responses may lead to performance failure of the implant. The current developments are towards integrating the implant to the body (72). Various surface modification techniques have been adopted to look the surface more natural. However least attempts have been made to manipulate the interphase. Few of the current techniques include endothelialization, coating the surface with adhesive proteins or peptides etc., are attempts in that direction. Again here the possibilities of immune rejection cannot be totally neglected. As the restoration of complete homeostasis is required for proper integration of the implant, it needs not to be two-way like in the case of fetus, but it should be able to form full circuit with the circulatory system. Porous matrix approach compounded by proper engineering principles to relax the applied stress, found to be the most promising approach towards achieving this goal in all the fields, right from vascular grafts to hip replacement. The tissue integration is regained in these porous structures, but at a slow pace.

The ability of the cord blood to reduce the immune reaction selects it as a good candidate for modifying the interface. The higher concentration of the Th2 cytokines may be the reason for the reduced activation of cord blood lymphocytes (73). This is observed as a reduced immune response in bone marrow transplantation using cord blood stem cells (74). This inefficiency could be used for modifying the interfacial immune reactions at an implant site, provided all the precautions are taken care off, as in bone marrow transplantation (75). The tolerogenic potential of the cord blood could be further improved by redirecting the cytokine profile with the help of specific bioactive signals., Eg: macrophage colony stimulating factor (76). This is because cord blood contains every primary requirement like growth factors, stem cells and immune suppressing chemokines for tissue regeneration.

Conclusion

We have reviewed the possibilities of using cord blood as a “cocktail” for modifying the interface of material-biology interaction for the fast integration of the implants with the neighbouring tissue. There is interesting correlation in immune profiles of foeto-maternal system and implant in host environment. The role of Th2 cytokines in regulating the maintenance of foeto-maternal system
could be mimicked using cord blood for implant integration. The tissue integration should sufficiently supported with sufficient blood vessels at the implant site (with proper homeostasis), as in the foeto-maternal system to avoid any necrosis and chronic inflammation. The growth factors present in the cord blood could be able to sufficiently enhance the angiogenesis at the implant site along with growth and integration of the neighboring tissue. The stem cells can be differentiated to any cell lineages according to the inductive stimuli at the implant site once the tissue regeneration is induced. The current strategy of implant-tissue integration in biomaterials is by utilizing the acute inflammatory reactions favorable, by modifying the surface properties of the implant. However, using cord blood the implant site could be introduced to a new environment of cocktail containing growth hormones, anti immune cytokines and stem cells for fast and effective regeneration and integration of the implant with the tissue.

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Establishment of the UK Stem Cell Bank and its Role in Stem Cell Science

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1. Introduction

The UK has administered an indepth and broad ranging debate on the use of human embryos for research, stretching over at least two decades, which led to the Human Fertilisation and Embryology Act of 1990. The Human Fertilisation and Embryology Authority was established in 1991 to regulate and license all applications to work with human embryos in the UK. Following the publication of the first human embryonic stem cell lines (Thomson et al., 1998) a Parliamentary House of Lords Select Committee was set up in 2001 to discuss the use of human embryos for research and the development of therapy as part of a broad public consultation. This process involved extensive dialogue between the public, politicians and other professional stakeholders. Reporting in June 2002 the Select Committee recommended that there should be a bank established to provide stem cell researchers with ready access to embryonic stem cell lines of guaranteed purity and provenance from sources with appropriate ethical approval. In the same year the Government endorsed the recommendations of the Select Committee report and announced that a National Stem Cell Bank would be established to curate and maintain stocks of somatic and embryonic stem cells, derived in the UK under the proposed regulation. Such cell lines would be prepared as quality-controlled cell banks, to enable ready access for researchers to reliable, high quality and ethically sourced stocks of these precious cells.
In order to establish the Bank the Medical Research Council (MRC) coordinated competitive
tenders from Institutions with appropriate facilities and experience but not engaged in basic stem
cell research. This selective process was important to ensure that the bank could operate as an
independent custodian of the cells without the conflict of interest in using the cells that might
occur in a centre for stem cell research. Following a lengthy evaluation process, in January 2003,
£2.6M was awarded to NIBSC for 3 years from MRC (75% of funds) and the Biotechnology and
Biological Sciences Research Council (25% of funds).

2. Aims and Remit for the UK Stem Cell Bank

The primary remit of the Bank is to provide access to ethically sourced and well characterised
seed stocks of human stem cell lines of both somatic (adult and foetal) and embryonic origin. The
Bank is also expected to work on an international collaborative basis with researchers and
other banking centres. Stem cell lines will be provided for two types of use: firstly “research
grade” banks will be prepared to promote basic research and secondly, “clinical grade” banks will
provide seed stocks for clinical trials and development of stem cell products.

3. Key Operational Principles for UK Stem Cell Bank

The Bank is required to maintain transparent operational procedures including publication of the
Code of Practice for the UK Stem Cell Bank (www.mrc.ac.uk) which describes how the bank can
be expected to operate and interact with the stem cell community and its responsibility to the
Steering Committee for the UK Stem Cell Bank and the Use of Stem Cell Lines.

Given its unusual role as an independent ‘broker’ in the stem cell community and to ensure that it
cannot be accused of conflicts of interest the Bank is prohibited from carrying out fundamental
research on stem cell biology although it is permitted to develop and enhance methods for
culture, preservation, characterisation and safety testing involved in the cell banking process. The
Bank is also prohibited from engaging in commercial activities and specifically product
development, although it is important that NIBSC and Bank staff are engaged with companies to
support their work to enhance the safety and quality of stem cell products.

The Bank has also made extensive efforts to develop a strong and close liaison with the stem cell
community. As a key part of NIBSCs work there is close interaction with numerous regulatory
bodies (approximately 80 world-wide) and this role is also developing for stem cell therapies as
part of the Bank and general NIBSC activity. The Bank must be sensitive to the broad range of
groups with interests in stem cells and their clinical potential, and also provides information for the press and media on the work of the Bank and its role in the stem cell community.

The high profile of the Bank means that it is focussed on reliability of its outputs and achieved this thorough careful proofing of its procedures and trying to anticipate and explore the implications of potential future developments. This is achieved through building flexibility within staff and facilities and close interaction with lead research groups regulators and clinical groups.

4. Donating Cells to the Bank

Embryonic stem cell lines established in the UK are required, under the licence for derivation from the Human Fertilisation and Embryology Authority, to be deposited in the UK Stem Cell Bank. Other groups working on the derivation of adult and non-UK hES Cells are also very welcome to use the UK Stem Cell Bank facility. Donation of cell lines into the Bank is initiated by submission of information on the lines to the Bank’s Steering Committee using the forms available on the Bank and Medical Research Council websites. Confirmation by the Steering Committee that the cells meet ethical requirements for the UK and other scientific and technical criteria then activates the depositing process with the Bank and establishment of the transfer agreements between depositor and the Bank. A generic agreement is also established to be put in place between depositor and any institution receiving cells from the Bank to protect the depositor’s intellectual property in the cells.

Once a depositor’s cell line is accepted for the Bank the depositor will hopefully then begin to realise a series of technical, logistical and other benefits that come with depositing cells in the UKSCB. These can include:

• Technical benefits -
  • Detailed characterisation and quality control of the cells
  • Enhanced technical procedures for cell culture, preservation and testing procedures

• Resource benefits -
  • The Bank takes on scale-up, quality control and distribution
  • The Bank provides an assured safe depository

• Potential economic benefits -
  • Added value in having cell banks that meet international quality standards thus aiding development both for clinical and testing applications of the cells
  • Safety testing regimes for clinical grade cell banks
• Promote wider use of depositors’ cells for research whilst protecting IPR under a Materials Transfer Agreement established by the depositor
• For the purposes of filing stem cell patents the Bank can also provide patent deposit facility (see below)

Stem cell lines released from the UKSCB must be used only in projects that have appropriate ethical review and approval. Requests to obtain cell lines from the Bank must be submitted to the Bank’s Steering Committee using the forms available on the Bank and Medical Research Council websites. This is a straightforward process of submitting a summary of the scientific and ethical information associated with the intended use of the cells to the UK Steering Committee. Early contact with the Bank is recommended to assist applications.

5. What is a Cell Bank?

The term “cell bank” has been applied generically to describe what is in fact a very broad range of entities. There are a wide variety of “tissue banks” established for cell and tissue transplantation for a range of tissues requiring different approaches and procedures. Whilst they all have a common need for donor consent and the need for donor medical histories and screening for viral markers these requirements will differ between countries. Public service culture collections (see www.eccosite.org and www.wfcc.info) provide cell banks but are customer focused and provide immortalised cell lines with fundamental quality control for a very broad range of uses in research and industry. Moving to another level pharmaceutical grade cell banking facilities are required to comply with Good Manufacturing Practices (cGMP) (MCA, 1992) and intensive quality control and safety testing where cells are used for product manufacture. The UK Stem Cell Bank for stem cell lines must combine all of these activities in its role as a public service collection for research and as a source of seed stocks for clinical trials.

6. Principle of Cell Banking

An important principle of banking for any micro-organism including cell lines is that there should be an early passage stock of viable cryopreserved cells that provide a primary source of material as an archive of the original material for future reference. This stock may be referred to as the Master Cell Bank and samples from this bank should be fully quality controlled and characterised. Ampoules from the master bank are recovered to produce expanded cultures at slightly higher passage level that can be cryopreserved as Working Cell Banks that may be used for R&D, production or clinical therapy. If prepared correctly, this tiered master/working bank system (Figure 1) can provide reproducible and reliable supplies of identical cultures for many decades.
7. Fundamental Criteria for Assurance of Quality of Cell Lines

There are three fundamental characteristics of a cell line required to promote good quality and valid cell culture work:

- Purity: absence of micro-organisms
- Authenticity: correct identity and absence of “other” cells
- Stability: passage in vitro and storage

In general most rigorous testing is performed on the Master Cell Bank but for both master and working stocks it is important to have a combination of quality control tests for all these features.
 whilst for extended cell banks of stem cell lines the main focus would be on the genetic/phenotypic stability of the cells in the undifferentiated state and their sustained ability to differentiate reproducibly (Stacey, 2005).

In Europe regulation of tissue engineering has begun to develop and in the UK guidance in this area has been in place for some time as Codes of Practice to provide guidance for tissue banks (DH, 2001), manufacturers of human tissue products (DH, 2002) and for the banking of stem cell lines (www.ukstemcellbank.org).

The general risk from endogenous contaminants of the cells or the original tissue of origin may be assessed from their tissue and species of origin. Trypsin and serum can be tested for likely contaminants or treated by irradiation, and there are also alternatives such as materials of plant or crustacean origin that may be used in place of animal proteins such as trypsin. In addition serum-free growth media can be used to avoid the risks of virus and mycoplasma contamination. However, it is important to recognize that some cells may not be amenable to the use of these alternatives and that their use may introduce new complexities or contaminants to the in vitro culture environment and moreover may alter certain characteristics of the cell lines.

Viral contamination may arise from the original tissue used to derive a cell line or from materials of animal origin used in the cell line derivation process as already indicated, and it is important to evaluate these risks. Important sources of information that promote safety of cell therapy products include: donor screening for viral markers, and risk assessment of material of animal origin. However, additional risk factors include contamination from cell culture operators, and endogenous viruses that may emerge from the host genome.

8 Embryonic Stem Cells in vitro: Control and Standardisation for Research and Therapy

S""em cells cultured in vitro are potentially unstable and highly sensitive to environmental variables with much of their cell biology yet to be determined. Nevertheless, if work with these cells is to progress there is a clear need for reliable and reproducible supplies of cells that perform consistently. At the UKSCB we aim to address this by: 1) attempting to minimise variation in culture environment, 2) establishment of a framework of controls and well defined culture reagents, procedures and quality control tests and 3) attempting to characterise the residual variation in the cultures.

Maintaining accurate measurement and control of the temperature and gaseous environment provided for cell culture is vital to these aims, as will be the use of reagents and media that are closely specified for their composition, purity and batch to batch reproducibility. Culture protocols will also be carefully captured from the laboratory of origin that will also be engaged in an ongoing
interaction to ensure that scientific and technical best practice are maintained as methods are improved. Further definition of the culture environment may be achieved as serum-free and feeder cell-free methods are developed. Such approaches have significant benefits in reducing the media variation and risk of certain types of viral contamination from culture reagents. However, the effect of such conditions on the performance of the cells must also be carefully monitored.

9. Stem Cell Lines for Clinical Use: Three Translational Phases

UKSCB provides a translational role taking research developments and attempting to translate these into starting materials (cell banks) that are appropriately qualified for use in clinical trial. The first phase is to capture a set of methodologies and protocols from the research environment that will give laboratory workers support in achieving technical proficiency. This role will also be very important for the development of protocols that can be used reliably in the product development and production settings for initiation of clinical trials.

In the next phase the Bank must establish robust and reliable banking procedures that may vary for different cell lines depending on their growth characteristics. A core quality control and safety testing regime will be applied to each bank and for each line a specification is produced to describe and quantify, wherever possible, the key characteristics to be maintained. This specification is used to assess the certificate of analysis compiled for each new cell bank of that line and to assess if the bank is of the appropriate standard for release.

In the final phase cell bank vials are transferred to researchers or clinical trials. In the former case obtaining feedback on the performance of the cells will be vital to maintaining the standards of the Bank and responding to customer difficulties. Cells released for clinical trials it is anticipated that there will be a period of iteration for the Bank and recipients to discuss the testing applied to the bank is appropriate for the clinical trial and if additional qualification of the cell line is required. In addition mechanisms will have to be established for recall procedures due to post-donation disease in donors and adverse event reporting from patients in the clinical trial.

10. Progress in Establishing the UK Stem Cell Bank

The establishment of the Bank has been a high profile activity due to the associated ethical concerns and high expectations of the public for stem cell research. Accordingly the Steering Committee for the Bank has taken a very careful approach to setting up its framework and the Bank has reflected this in developing a robust and thoughtful approach its establishment and operation. Appropriate time has been allocated to these activities and to the physical construction of the Bank. In 2003 four new core staff were recruited, facilities for research grade
cells were established, a GMP compliant facility was constructed and a Code of Practice describing how the Bank should operate was published on the Medical Research Council website.

In 2004 the Bank achieved accreditation to provide cells for clinical use from the Medical and Healthcare products Regulatory Authority (MHRA). The Steering Committee approved the first lines for the Bank in May 2004 (the first two hES line derived in the UK at Kings College and Newcastle Centre for Life) and in December a further 22 hES cell lines from the UK, the USA and Australia were approved. The Bank also achieved patent depositary status in 2004 which is recognised by the World Intellectual Property Organisation. Currently the staff comprises a total of nine scientists including 4 PhDs and a quality manager with Qualified Person experience for release of medical products. Since 2003 the Bank has also been involved in training activities for culture of hES cells with Professors Harry Moore and Peter Andrews at the University of Sheffield and more recently with a number of other organisations worldwide. In 2004-2005 the Bank provided the hub for the International Stem Cell Initiative (see below) and is now banking the remaining cell lines approved by the Steering Committee.

11. UK Stem Cell Bank/NIBSC Interactions with the Stem Cell Community

The staff at NIBSC have been engaged with the transplantation community for many years and have coordinated a liaison group on haematopoietic stem cells for a number of years (see http://www.nibsc.ac.uk/aboutus/ukscbliaison.html). The Bank has engaged directly with stem cell biologists in many countries and has presented at scientific conferences on stem cells and regenerative medicine conferences around the world. The Bank has also provided the hub for the Medical Research Council led project called the International Stem Cell Initiative. This project headed by Professor Peter Andrews of Sheffield University aims, through a carefully standardised process, to compare the characteristics of around 70 hES cell lines in 17 expert research centres around the world (Andrews et al., 2005). Details of this activity can be found at http://www.stemcellforum.org/ and the results of the whole project will be published in 2006.

12. Conclusions

Whilst stem cell lines have the potential to deliver an exciting new range of therapies there are still important issues to address to establish safe and efficacious cell therapy products. There is still considerable research to be done on the basic culture and differentiation processes required for the development of therapeutic applications. The UK Stem Cell Bank aims to support the stem cell community at various levels: providing banking facilities and collaboration on technical, safety
issues and training. The NIBSC, the host institution for the UK Bank, has a background and long-standing experience in the field of biological medicines which enables it to provide an advisory role free of conflicts of interest. The Bank is therefore well placed to help set standards for safe and reliable stem cell therapies as they are developed and provide a valuable advisory role in the stem cell field.

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Cord blood transplantation for hematologic malignancies

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The first human cord blood transplant was performed in 1988 by Dr. Gluckman and colleagues in Paris; a 5-year-old boy with Fanconi’s anemia received his HLA-identical sister’s cord blood that had been cryopreserved.¹ This patient is disease-free more than 15 years later with complete donor chimerism.

Many children have subsequently received cord blood transplants from related donors (generally a sibling), for a variety of conditions such as marrow failure syndromes, congenital immunodeficiencies, metabolic disorders, hemoglobinopathies, hematologic malignancies, and solid tumors. Cord blood transplants have two main advantages over marrow or peripheral blood stem cell grafts in the related donor setting: first, cord blood collection is risk-free for the donor, avoiding the need for general anesthesia (in the case of marrow harvest) or growth factor mobilization and leukapheresis (in the case of peripheral blood stem cell harvest); second, a cord blood transplant is generally considered to have a lower risk of graft-versus-host disease (GVHD) than a conventional (unmanipulated) marrow graft.

A retrospective comparison of 113 cord blood transplant and 2052 marrow transplants in children (15 years of age or younger) for the period 1990-1997, all from HLA-identical sibling donors, was performed through the International Bone Marrow Transplant Registry and Eurocord.² Over half of the patients were transplanted for malignancy, most commonly acute leukemia. Recipients of cord blood were younger (median age of 5 years vs. 8 years) and smaller (median weight of 17 kg vs. 26 kg.) than recipients of bone marrow. The median cell dose was 4.7 x 10E7 cells/kg for cord blood, compared to 3.5 x 10E8 cell/kg for marrow. Despite less intense GVHD prophylaxis (with the omission of methotrexate in 72% of cord blood recipients), cord blood transplants had a lower risk of acute GVHD (14% vs. 24%) and chronic GVHD (5% vs. 14%). Hematopoietic recovery in terms of neutrophil (26 vs. 18 days) and platelet (44 days vs. 24 days) engraftment was significantly slower for cord blood transplantation compared to marrow. Three-year survival in patients transplanted for malignancy was similar in the two groups (46% for cord blood recipients, 55% for marrow recipients).
Only 30% of patients have an HLA-identical sibling donor. Some of the patients who do not have an HLA-matched related donor may find a matched unrelated marrow donor. For those who do not find an acceptably matched unrelated marrow donor, a mismatched unrelated cord blood transplant is a viable option. The search and procurement process for an unrelated cord blood graft through a cord blood bank is generally much faster (under one month) than the comparable process for an unrelated marrow donor (3-4 months), since the cord blood graft is already collected.25

The New York Blood Center operates the oldest public cord blood bank in the United States and reported on its experience providing unrelated cord blood grafts for 562 patients in the U.S. and abroad for the period 1992-1998.26 HLA-A and B antigens were determined serologically, HLA-DRB1 alleles were determined by high-resolution DNA typing. Eighty-six percent of cord blood grafts were one or two-antigen mismatches with the transplant recipient. Two-thirds of patients were under 12 years of age or weighed less than 40 kg. Over 80% of patients were under 18 years of age or weighed less than 60 kg. Three-quarters of patients had hematologic malignancies, mainly acute leukemia in an intermediate or advanced stage of the disease, or acquired bone marrow disorders such as myelodysplastic syndrome or aplastic anemia. The remaining one-quarter of patients had genetic diseases such as Fanconi's anemia or severe combined immunodeficiency.

Myeloid engraftment post transplantation (absolute neutrophil count ≥ 500/cu mm) occurred at a median of 28 days. Successful myeloid engraftment (by day 42) was significantly associated with a higher cell dose (>2.4 x 10⁷ nucleated cells/kg) and the absence of HLA mismatching in a multivariate analysis. Platelet engraftment post transplantation (platelet count ≥ 50,000/cu mm) occurred at a median of 90 days. In multivariate analysis, age, infection after transplantation, and GVHD were significant factors affecting platelet engraftment.

The incidence of acute GVHD was almost 70%; about one-third of GVHD cases were grade III or grade IV. In multivariate analysis, age and HLA mismatch were independently associated with acute GVHD. Mortality at day 100 was 39%; the deaths were attributed to infection, respiratory failure, multiorgan failure or GVHD. This figure was felt to compare favorably with bone marrow transplantation from unrelated donors, especially considering that two-antigen mismatches are generally not accepted in unrelated marrow transplantation. The conclusion of this report was that umbilical cord blood is a useful source of allogeneic hematopoietic stem cells for bone marrow reconstitution.

Eurocord is an international registry operating on behalf of the European Group for Blood and Marrow Transplantation (EBMT); it includes more than 180 transplant centers worldwide in 35 countries, all performing cord blood transplants. A retrospective analysis comparing the outcome of unrelated cord blood transplants to unrelated marrow transplants in children with acute leukemia for the period 1994-1998 was reported by Eurocord and EBMT.4 There were 99
unrelated cord blood transplants (UCBT), 262 unmanipulated unrelated bone marrow transplants (UBMT), and 180 T-cell depleted unrelated bone marrow transplants (T-UBMT). The UCBT patients were younger (median age of 6 years), and contained a higher proportion of patients with AML (30%). Fourteen percent of UCBT patients had undergone prior transplants for relapse (12 autologous, 2 allogeneic transplants). The median nucleated cell dose for UCBT was 3.8 x 10^7/kg (range of 2.4-36 x 10^7/kg), one log less than that for UBMT or T-UBMT. HLA-A and B antigens were determined serologically; HLA-DRB1 alleles were determined by high-resolution DNA typing. Most UBMTs were 6/6 matches (81%); most T-UBMTs were 6/6 matches (54%) or one-antigen mismatched (34%). Most UCBTs were one-antigen mismatched (43%) or two-antigen mismatched (41%).

Myeloid engraftment post transplantation (absolute neutrophil count ≥ 500/cu mm) occurred at a median of 32 days for UCBT, compared to 18 days for UBMT and 16 days for T-UBMT. Platelet engraftment post transplantation (platelet count ≥ 20,000/cu mm) occurred at a median of 81 days for UCBT, compared to 29 days for both UBMT and T-UBMT. Treatment-related mortality at day 100 was significantly higher in the UCBT group (39%) compared with the other two groups (19% for UBMT and 14% for T-UBMT). The incidence of acute graft-versus-host disease and the incidence of grades III/IV GVHD at day 100 was 35%/22% for UCBT, 58%/30% for UBMT, and 20%/8% for T-UBMT. The incidence of chronic GVHD was 25% for UCBT, compared to 46% for UBMT, and 12% for T-UBMT. A lower incidence of GVHD raises the theoretical concern that a diminished graft-versus-leukemia effect might lead to a higher relapse rate. In this study, relapse at 2 years was the same (38%) for both UCBT and UBMT, but 47% for T-UBMT. Survival at 2 years was 35% for UCBT, compared to 49% for UBMT and 41% for T-UBMT. The conclusion of this report is that for children with acute leukemia who lack a matched sibling donor, a simultaneous search of bone marrow donor registries and cord blood banks should be done. The final choice for stem cell source balances donor-recipient histocompatibility with the urgency of the transplant, and the cell dose of the cord blood unit.

A subsequent Eurocord analysis focused exclusively on the role of cord blood transplantation (UCBT) in the treatment of relapsed or high-risk acute myeloid leukemia (AML) in children. Many children with relapsed AML who will potentially benefit from bone marrow transplantation do not have an HLA-matched sibling donor or unrelated donor, or the search for an unrelated donor takes too long. UCBT can be performed safely with up to two-antigen mismatches, and the search process is much faster. This study identified 95 children with AML who underwent UCBT between 1994 and 2002 in 17 countries. The median age at transplantation was 6 years. Ten percent of patients had secondary AML (excluding patients with Fanconi's anemia). Half the patients were in second complete remission (CR2), 29% of patients were high-risk by virtue of being in relapse or an advanced CR (beyond CR2), and 21% were in first complete remission (CR1). Most of this latter group had unfavorable cytogenetic
abnormalities such as monosomy 5 or 7 or 11q23 abnormalities, or had secondary leukemia. Twenty-two patients had undergone prior hematopoietic stem cell transplants (18 autologous transplants in relapse, 4 unrelated marrow transplants with engraftment failure). HLA-A and B antigens were determined serologically; HLA-DRB1 alleles were determined by high-resolution DNA typing. Eighty percent of grafts were mismatched for one or two antigens; 11% were mismatched for 3 or more loci. The median collected cell dose was 5.2 x 10E7/kg. Significantly, reflecting more recent UCBT practice, half of patients received a hematopoietic growth factor in the early post-transplantation period.

Myeloid engraftment occurred at a median time of 26 days; 6 patients did not have neutrophil recovery by day +60. In multivariate analysis, the factors associated with neutrophil recovery were status of disease at transplantation (CR1 or CR2 were favorable) and prophylactic use of hematopoietic growth factor. Platelet engraftment occurred at a median time of 52 days (platelet count ≥ 20,000/cu mm.); 5 patients did not have platelet recovery by day +180. The only significant factor associated with platelet recovery was disease status at the time of transplantation. The incidence of acute graft-versus-host disease and the incidence of grades III/IV GVHD by day 100 were 35% and 20% respectively. The incidence of chronic GVHD at two years was 15%. Treatment-related mortality (TRM, defined as all causes of nonleukemic deaths occurring after transplantation) was 20%; most of these deaths were caused by infection. In multivariate analysis, TRM was associated with a low collected nucleated cell dose (less than 5.2 x 10E7/kg). Overall relapse incidence at 2 years was 29%. Not surprisingly, advanced disease at the time transplantation (CR3 or higher or no CR) was associated with relapse: the relapse rate was 61% for patients who were not in remission at the time of UCBT. Overall survival and leukemia-free survival at 2 years were 49% and 42% respectively. Both these outcomes were significantly associated in multivariate analysis with disease status at the time of UCBT, and interestingly, by major ABO incompatibility. UCBT occurring after 1997 was significantly associated with leukemia-free survival in univariate analysis.

The conclusions of this study were three-fold. First, leukemia-free survival after UCBT for childhood AML is associated with disease status at the time of transplantation; relapse incidences for the different stages of disease are felt to be comparable to those reported after unrelated bone marrow transplantation. Second, the outcome of UCBT is not influenced by three traditional prognostic factors of childhood AML: unfavorable cytogenetics such as monosomy 5 or 7, secondary AML from prior chemoradiation or bone marrow stem cell disorder (such as myelodysplastic syndrome), and the duration of first CR for patients transplanted in CR2. This seems to support a potent graft-versus-leukemia effect for these subsets of traditionally poor-risk factors. Finally, treatment-related mortality (TRM) is significantly influenced by an adequate nucleated cell dose: above the median collected cell dose of 5.2 x 10E7/kg, TRM was 9%, much lower than the overall TRM of 20%. Other studies have suggested that the impact of cell dose is
even more significant with increasing HLA disparity (see later section). The effect of cell dose is correlated with infection as the major contributor to non-relapse mortality; this has implications for the prophylaxis and diagnosis of infection in UCBT.

A recent Eurocord update of 323 pediatric patients with acute lymphoblastic leukemia (ALL) who underwent UCBT for the period 1994-2004 was reported. The median age was 6.5 years at UCBT; median cell dose infused was $4.1 \times 10^7$ cells/kg. Eighty-five percent of cord blood grafts were one- or two-antigen mismatches. Forty-two percent of patients ($n=136$) were transplanted in second complete remission (CR2); thirty-four percent of patients ($n=111$) were transplanted for advanced disease and 20% of this group had undergone prior autologous transplantation; the remainder were transplanted in first complete remission (CR1) and 89% of this group in CR1 had poor-risk cytogenetics. Leukemia-free survival at 2 years was 36% for the entire cohort. In multivariate analysis, CR1 and CR2 were associated with better leukemia-free survival (41-42% for these two groups compared to 24% for the patients with advanced disease). The conclusion of this analysis is that UCBT should be offered for children lacking an HLA-identical donor, in an earlier disease state.

Another recent comparison of 508 children with acute leukemia who underwent UCBT and 492 children who underwent unrelated marrow transplantation (UBMT) in the United States for the period 1995-2003 was reported on behalf of the New York Blood Center and the IBMTR. This study makes a very strong case for the superiority of matched cord blood over matched marrow, and illustrates the interaction of cell dose with HLA disparity. A high cell dose for UCBT was defined as greater than $3 \times 10^7$ cells/kg. There was no difference in neutrophil and platelet engraftment between the matched marrow recipients and the matched UCBT recipients. For the recipients of mismatched UCBT, cell dose was significantly associated with neutrophil and platelet recovery. The incidence of acute and chronic GVHD was lower for UCBT, matched and mismatched, compared to UBMT. Transplant related mortality was lowest for matched UCBT at 6%, compared to TRM for matched UBMT at 26%. For one-antigen mismatched UCBT, cell dose had a major impact on TRM: TRM was 29% for a high cell dose, compared to 43% for a low cell dose. For two-antigen mismatched UCBT, TRM was the highest at 47%. Leukemia-free survival (LFS) at 3 years was highest for matched UCBT at 60%; LFS was the same for one-antigen mismatched UCBT with a high cell dose and matched bone marrow (40-41%). Relative to matched UBMT, matched UCBT had lower TRM and superior LFS and overall survival (OS), one-antigen mismatched UCBT with a high cell dose had similar TRM, LFS and OS; one-antigen mismatched UCBT with a low cell dose and two-antigen mismatched UCBT had higher TRM and lower LFS and OS. The conclusion of this study was that for pediatric patients with acute leukemia who are candidates for hematopoietic stem cell transplantation, a one-antigen mismatched UCBT with a high cell dose or a matched UCBT is preferable to matched UBMT.
Japan has successfully developed a network of cord blood banks in recent years. It has reported on 411 cord blood transplants for malignancies for the period 1997-2001, mostly in children. However, unlike the Eurocord or New York Blood Bank/IBMTR data, a much higher proportion (65%) of UCBT were matched or one-antigen mismatched. Disease-free survival at 3-years was 35%; cell dose but not HLA disparity was associated with survival.

In conclusion, sufficient data exist to support comparable efficacy between HLA-matched unrelated marrow and matched or one-antigen mismatched UCB with an adequate cell dose for pediatric patients with acute leukemia. The minimum cell dose is not precisely defined, but should be around 2.5-3.0 x 10E7 nucleated cells/kg. In one study CD34 cell dose (greater than 1.7 x 10E5 cells/kg) was the one factor significantly associated with rate of engraftment, treatment-related mortality, and survival.

**Cord Blood Transplantation in Adults**

In recent years, the use of UCBT in adults with hematologic malignancies has increased. Eurocord and the European Blood and Marrow Transplant Group performed a retrospective analysis of 682 adult patients with acute leukemia who had undergone UCBT (n=98) or matched unrelated marrow (UBMT) transplant (n=584) with myeloablative conditioning for the period 1998-2002. The UCBT patients were younger (median age 24.5 years vs. 32 years) and weighed less (median weight 58 kg. vs. 68 kg.) than the UBMT patients. More UCBT patients had advanced disease (52% beyond CR2, compared to 34%) at the time of transplantation, or had undergone a prior autologous transplant (19% vs. 8%). Ninety percent of UCBT were one or two-antigen mismatches defined by serologic typing or low-resolution DNA typing for HLA-A and B, and high-resolution DNA typing for HLA-DRB1. The median cell dose was 2.3 x 10E7 nucleated cells/kg for UCBT recipients, compared to 2.9 x 10E8 cells/kg for UBMT recipients. The median number of CD34+ cells in the cord blood grafts was 1.1 x 10E5 cells/kg. Consistent with the Eurocord pediatric series, 77% of UCBT recipients received antithymocyte or antilymphocyte globulin as part of their conditioning, compared to 37% of UBMT patients. The predominant GVHD prophylaxis regimen was cyclosporine and corticosteroids (70%) in the UCBT patients, and cyclosporine and methotrexate (95%) in the UBMT patients. Data were not given on the use of post-transplant hematopoietic growth factors.

Myeloid engraftment was delayed in UCBT recipients, with the median time to neutrophil recovery occurring at day 26, compared to day 19 for UBMT recipients. The incidence of graft failure was high in this series: 7% in the marrow group and 20% in the cord blood group. The incidence of GVHD (grades II-IV) by day 100 was significantly lower in the UCBT group (26% vs. 39%). The two-year cumulative incidence of chronic GVHD was 30% for UCBT and 46% for UBMT (not significant). There was no difference in transplant-related mortality (TRM, defined as all causes of nonleukemic deaths occurring after transplantation): 44% for UCBT and 38% for
UBMT. However, causes of TRM were predominantly infections or toxicity for UCBT, and infections or GVHD for UBMT. Relapse was associated with advanced disease; the rate was 23% for both groups. Leukemia-free survival and overall survival were also similar between the two groups: 33%/36% for UCBT and 38%/42% for UBMT. The conclusion of this study is that mismatched UCBT for acute leukemia in adults is a viable alternative to matched UBMT. Relative to UBMT, UCBT is associated with delayed myeloid recovery, but a lower incidence of acute GVHD despite HLA disparity. Both these outcomes are similar to the pediatric experience. Unlike the pediatric experience reported by the same group, transplant-related mortality was not higher for UCBT compared to UBMT, despite delayed neutrophil recovery which carries a high risk of infection. The fact that this adult series included transplants in a later period (1998–2002) probably points to improved experience managing prolonged neutropenia.

Another recent retrospective analysis of UCBT in adults paints a slightly less promising picture. Data on adult patients who underwent UCBT (one or two HLA mismatches) through the national cord blood program of the New York Blood Center or unrelated marrow transplants (0 or 1 HLA mismatch) through the IBMTR for the period 1996–2001 in the United States were collected. There were 367 recipients of matched bone marrow (UBMT), 83 recipients of one-antigen mismatched bone marrow (UMBMT), and 150 recipients of one or two-antigen mismatched cord blood (UCBT). Patients who had undergone prior transplantation were excluded. UCBT patients were younger (median age around 30 years) and weighed less (median weight 68 kg.) Unlike the Eurocord study above, hematologic diagnoses other than acute leukemia were included; specifically, the marrow recipients had a much higher proportion of patients with chronic myelogenous leukemia (CML) compared to the UCBT recipients (40% UBMT, 45% UMBMT, 25% UCBT). Similarly, the UBMT group had a higher proportion of good-risk patients (first complete remission, first chronic phase for CML, and refractory anemia): 40% for the UBMT group, 33% for the UMBMT group, 20% for UCBT. Conversely, the cord blood recipients had a much higher proportion of advanced disease (relapse, primary induction failure, CML blast crisis, or secondary AML from myelodysplastic syndrome): 43% for UCBT, 29% for UBMT, and 25% for UMBMT. The median cell dose was 2.2 x 10^7 cells/kg for the UCBT group, compared to 2.2-2.4 x 10^8 cells/kg for the marrow recipients.

Consistent with prior studies, median times to neutrophil recovery were significantly longer in UCBT patients (27 days UCBT, 20 days UMBMT, 18 days UBMT). Median times to platelet recovery (platelet count > 20,000/cu. mm.) were 60 days in UCBT, and 29 days for both marrow groups. The incidence of acute GVHD was highest in the UMBMT group at 52%, and similar between the UCBT and UBMT groups (41% and 48% respectively). The incidence of chronic GVHD was highest in the UCBT group at 51%, compared to 40% in the UMBMT group and 35% in the UBMT group, although the proportion of patients with extensive chronic GVHD was lowest in the UCBT group. Treatment related mortality (all non-relapse causes of death) was
lowest for UBMT at 46%, compared to 65% in the UMBMT group and 63% in the UCBT group. Relapse rates were similar in the three groups (17% UCBT, 14% UMBMT, 23% UBMT). Leukemia-free survival at 3 years was significantly higher for UBMT at 33%, compared to 23% for UCBT and 19% for UMBMT; as expected, leukemia-free survival was associated with age and disease status. Overall survival at 3 years was 35% for UBMT patients, compared to 26% for UCBT patients, and 20% for the UMBMT group. The conclusion of this study is that in the absence of a matched unrelated marrow donor, a one-antigen mismatched marrow graft or cord blood mismatched for one or two antigens are acceptable alternatives and have similar outcomes. The treatment related mortality in this series appears high, even for the matched marrow recipients whose TRM was 46%, considering that 40% of this group of UBMT patients had good risk disease (CR1, CML first chronic phase, or refractory anemia).

Other recently published series support the notion that the outcome of UCBT in adults varies greatly. The Cord Blood Transplantation study group in the U.S. \(^{12}\) reported on the adult subset of a prospective study of UCBT. Enrollment required a minimum of 1 x 10E7 nucleated cells/kg in the cord blood graft, and no more than two HLA mismatches at HLA-A and B (low or intermediate resolution DNA typing) and DRB1 (high resolution DNA typing). Thirty-four subjects with a median age of 35 years were entered. Most patients had a two-antigen mismatched graft. Diagnoses were predominantly acute leukemia (28/34 patients); most patients were poor risk by National Marrow Donor Program criteria. The primary end point was survival at 180 days; the result was 30%.

In contrast, one single-institution study in Japan is much more promising. \(^{13}\) This was a retrospective comparative analysis of 45 adults who received matched unrelated marrow transplants (UBMT) and 68 adults who received one or two-antigen mismatched cord blood transplants (UCBT) for the period 1997-2003. Median age of the UCBT group was 36 years, median weight 55 kg. More patients in the UCBT group had AML (57% vs. 33%); of the AML patients who received UCBT, more than half had advanced disease (beyond CR2). More patients in the UBMT group had CML (40% vs 7%); 60% of CML patients who underwent UBMT had advanced disease. All UCBT patients received prophylactic G-CSF. Median cell dose was 2.5 x 10E7 cells/kg for the UCBT group, and 3.3 x 10E8 cells/kg for the UBMT group. The UBMT group was HLA-matched (87%) or one-antigen mismatched (13%). Three-quarters of the UCBT group were one- or two-antigen mismatched; one-quarter was three or four-antigen mismatched. Despite slower neutrophil (22 vs. 18 days) and platelet (40 vs. 25 days) recoveries in UCBT patients, they had lower treatment-related mortality at 1 year (9% vs. 29%) and superior disease-free survival at 2 years (74% vs. 44%) compared with UBMT patients. Of note, relapse or refractory disease was the biggest contributor to UCBT deaths, not infection as in other series.

Japan has developed a national cord blood transplantation program within a relatively short period of time with excellent results. The relative HLA homogeneity in this island country
compared to the tremendous HLA diversity in a country such as the United States may play a role in these disparate outcomes.

In summary, UCBT in adult leukemia patients with an adequate cell dose is a viable alternative cell source for hematopoietic stem cell transplantation in the absence of an HLA-matched family or unrelated donor. Advances to overcome the limitation of cell dose especially in the setting of HLA disparity in the future will extend this option to a greater number of adult patients with hematologic malignancies or other acquired bone marrow disorders such as myelodysplastic syndrome.

**New Strategies in Cord Blood Transplantation**

Since an adequate cell dose from a single cord blood unit limits its wide applicability to adults, investigators explored the safety and efficacy of using two cord blood units to augment cell dose in high-risk adults and adolescents with hematologic malignancies. Initially, patients were eligible for double-unit UCBT if no single unit containing at least 2.5 x 10E7 cells/kg was identified. Subsequently, eligibility was broadened to include patients who did not have a single unit containing at least 3.5 x 10E7 cells/kg. The largest available UCB unit (at least 1 x 10E7 cells/kg) that was 4-6 antigen matched to the recipient was selected (UCB #1). Then UCB #2 (at least 0.5 x 10E7 cells/kg) was selected to be 4-6 antigen matched to the recipient and to UCB #1. HLA disparity between each unit and the recipient, and between the two units, were not necessarily at the same loci. Twenty-three patients were included in this report, with a median age of 24 years and a median weight of 73 kg. All had acute leukemia considered to be at high risk of relapse, except one patient who had CML in chronic phase refractory to standard treatment. The median infused cell dose was 3.5 x 10E7 cells/kg combined from both UCB units. The median CD34+ cell dose was 4.9 x 10E5 cells/kg. Double-unit infusion was well tolerated. All patients received G-CSF post infusion.

All 21 evaluable patients had sustained neutrophil engraftment at a median of 23 days; no patient had secondary graft failure. Hematopoiesis on day 21 bone marrow analysis showed 100% single-donor chimerism in 16/21 (76%) patients and dual-donor chimerism in the remainder; by day 100, all patients (17/17 alive) had single-donor chimerism. The graft factor predictive of engraftment was not the nucleated cell dose or the CD34+ cell dose, but a higher CD3+ cell dose. Of the 8/21 patients who received 2 UCB units with different degrees of HLA disparity, the better HLA-matched unit predominated in 4 patients, while the lesser matched unit predominated in 4 patients. The incidence of grades II-IV GVHD and grades III-IV GVHD was 65% and 13% respectively. Cumulative incidence of chronic GVHD was 23%. Transplantation-related mortality at 6 months was 22%. Major causes of death were infection or relapse; no patient died of GVHD. Leukemia-free survival at 1 year was 57%, with the major predictor being disease status at the time of transplantation. The conclusion of this study is that double-unit
UCBT produces durable single-donor engraftment without any apparent excess of GVHD. A higher CD3+ cell dose determined which unit predominated, which supports the hypothesis that donor predominance is immune-mediated. This strategy enables many more adult patients to have access to UCB grafts (graft made up one or two units) containing an adequate cell dose.

Another approach towards achieving an adequate cell dose is to use ex-vivo expansion of UCB cells. A phase 1 trial showed that one particular device was able to expand nucleated cells in UCB 2.4 fold but expansion of CD34+ cells was less successful. Twenty-eight patients enrolled in the trial, of whom only three were over 60 kg. The expanded cells were infused on day 12 and infusion was well tolerated. However, augmentation of UCB transplants with ex vivo-expanded cells did not alter the time to myeloid or platelet engraftment in 21 evaluable patients. The inability to expand primitive stem cells (CD34+lin-) is of some concern, although it is possible this could be improved with a different combination of early-acting cytokines. It is also not possible with this scheme to detect if the expanded cells are contributing to hematopoiesis since they were derived from the original UCB graft.

Along similar lines, other investigators have used low dose peripheral blood CD34+ cells from a related HLA-haploidentical donor with a primary unrelated umbilical cord blood graft. Eleven adult patients with poor-risk acute leukemia who did not have an HLA-matched donor (related or unrelated) or UCB unit with more than 4.0 x 10E7 cells/kg were studied. Median age was 23 years, and median weight 66 kg. The cord blood grafts were up to two-antigen mismatched containing a median infused cell dose of 2 x 10E7 cells/kg, and a median CD34+ cell dose (available for 7/11 units) of 1.1 x 10E5/kg. Haploidentical donors were siblings who shared a paternal HLA haplotype with the patient (n=6), the mother (n=4) and one father. The haploidentical CD34+ cells were obtained from the family donor by G-CSF mobilization, apheresis, and positive selection, then cryopreserved; the median CD34+ dose of haploidentical cells was 2.3 x 10E6 cells/kg.

Two patients died before myeloid engraftment: one from Grade IV GVHD, one from multiorgan failure; both had received maternal haploidentical cells. The other 9 patients reached an absolute neutrophil count > 0.5 x 10E9/L at a median of day 11: in 7/9 patients, neutrophils and mononuclear cells were initially primarily of haploidentical derivation, with subsequent shift to the cord blood genotype; the remaining two patients, both of whom received maternal haploidentical cells, achieved myeloid engraftment with full chimerism of CB cells on days +20 and +36. Complete chimerism exclusively of cord blood cells developed in 8 patients, at a median of 30 days. Platelet engraftment (platelet count > 20,000/ cu mm) occurred at a median of 43 days. Acute GVHD occurred in 6/11 patients (55%) with one death directly attributable to grade IV GVHD. Disease free survival at 3 years was 40%. CMV infection caused half the deaths. This study achieved the goal of shortening the period of neutropenia (compared to a single unit cord blood transplant) with neutrophils derived from the haploidentical CD34+ cells. A haploidentical
donor is easily available within the patient's family. There is a suggestion that acute GVHD may be potentially more severe with this approach compared to the double-unit cord blood transplant strategy.

In peripheral blood stem cell transplantation, there has been recent interest in the use of non-myeloablative or “reduced-intensity” preparative regimens to reduce regimen-related toxicity and therefore broaden transplant eligibility to older patients with co-morbid medical conditions that may preclude the use of myeloablative conditioning. The key component of such an approach rests on the graft-versus-malignancy effect, which takes time. Several regimens are widely used with varying degrees of myelotoxicity and immunosuppression. Most protocols are exploring the use of such regimens in diseases that have a slower rate of progression (for example, low-grade lymphoma and not acute leukemia) and for which myeloablative allogeneic stem cell transplantation is not the standard of care.

Published experience with non-myeloablative chemotherapy regimens in unrelated cord blood transplantation has been limited to small series. Most have been adults with a median age around 50 years. The diagnoses have included poor-risk leukemias, non-Hodgkin's lymphoma, Hodgkin's disease, and myelodysplastic syndrome. Engraftment ranges from 40% with a fludarabine/cyclophosphamide/antithymocyte-globulin regimen and a median nucleated cell dose of $2 \times 10^7$ cells/kg, to 91% for a fludarabine/cyclophosphamide/total body irradiation (200 cGy) regimen (Flu/Cy/TBI) and a median cell dose of $3.2 \times 10^7$ cells/kg mostly achieved with double-unit cord blood grafts. Most cord blood units were HLA-mismatched at one or two loci. GVHD prophylaxis consisted of cyclosporine and mycophenolate for most patients. The incidence of acute GVHD was relatively low, 44% in the Flu/Cy/TBI cohort. Disease-free survival at 1 year was 41% for this particular cohort ($n=22$, 15 of whom received double-units). The major contributor to treatment-related mortality was infection. Total nucleated cell dose and CD34+ cell dose were not associated with differences in survival in this series. The role of non-myeloablative regimens in cord blood transplantation merits further exploration.

High-resolution HLA typing by sequencing for HLA-A, B, C, DR, DQ has become available recently. To determine the impact of high-resolution (HR) HLA typing with outcomes after UCBT, DNA of 122 unrelated cord blood graft/recipient pairs were analysed for mismatches, and compared to the data from the traditional determination of HLA disparity (HLA-A, B on low-resolution typing, and HLA-DRB1 on HR typing). By the traditional approach, 13% were fully matched, 40% were one-antigen mismatched, 36% were two-antigen mismatched, 8% were three-antigen mismatched, and 3% were four antigen-matched. By the HR approach, 4% were fully matched, 10% were one-antigen mismatched, 15% two-antigen mismatched, 22% three-antigen mismatched, 25% four-antigen mismatched, 12% five-antigen mismatched, 6% six-antigen mismatched, 5% seven or eight antigen mismatched. Remarkably, there was no significant correlation between the number of HR mismatches on the one hand, and acute GVHD
grade II-IV and 2-year survival on the other. However, HLA-A locus mismatches on HR typing analysed in the host versus graft direction was associated with reduced cumulative incidence of engraftment. Killer-cell immunoglobulin-like receptor (KIR) incompatibility for HLA-C in the host versus graft direction was also significantly associated with impaired engraftment. Although the role of HR typing in UCBT remains to be defined, it is unlikely to change current practice until the donor pool of cord blood units enlarges.

**Conclusion**

Unrelated cord blood transplantation has a well established role in pediatric patients with hematologic malignancies: matched or one-antigen mismatched grafts are equivalent if not superior to matched unrelated marrow in this setting. Of course, the power of cord blood transplantation is the ability to transplant across greater HLA barriers. Two-antigen mismatched grafts produce very encouraging results for children who are transplanted in an early disease state (but have high-risk factors such as unfavorable cytogenetics).

An adequate cell dose is critical to the outcome of cord blood transplantation. Recent practice includes a nucleated cell dose of $2.5 \times 10^7$ cells/kg, or a CD34+ cell dose of $1.7 \times 10^5$ cells/kg. Cell dose appears to be particularly important in the setting of greater HLA disparity.

More UCBT has been performed in adults in recent years as the availability of larger banked cord blood units has improved. As with all UCBT studies, prospective randomized trials are not possible. Two recent retrospective comparisons of UCBT and unrelated marrow transplantation have somewhat different outcomes, but both support the notion that an UCBT with one or two antigen mismatches and an adequate cell dose is a viable option for adults with hematologic malignancies and no matched marrow donor. One single-institution report from Japan has particularly impressive results but may not be reproducible in another country given Japan's unique population HLA makeup (the same background that led to a high incidence of transfusion-related graft versus host disease in earlier literature).

Institutional experience is important because prolonged neutropenia and delayed immune reconstitution are expected for UCBT. One single-institution retrospective analysis showed that overall infection rates were higher in UCB recipients compared to recipients of matched unrelated marrow (or peripheral blood stem cells), particularly before day 50, when gram positive bacteremias predominated. In a larger Eurocord analysis of 510 UCBTs for the period 1994-2002, the incidences of overall, bacterial, viral and fungal infections were 69%, 49%, 32% and 10%. Shorter time to engraftment and UCBT performed after 1998 decreased the risk of all three types of infection in multivariate analysis. CMV seropositivity and HLA disparity (more than 3-antigen mismatches) were additionally associated with viral infection. Older age (> 16 yr) and presence of grades III/IV GVHD were additionally associated with fungal infections. Prevention and management of infection are clearly very important areas of clinical study.\textsuperscript{21,22}
New strategies such as double-unit cord blood transplantation or co-transplantation with haploidentical CD34+ cells or mesenchymal stem cells will help to shorten the period of cytopenias, elucidate the mechanisms of cord blood engraftment and broaden the applicability of UCBT to more patients especially adults. Pathophysiologic mechanisms of a preserved graft-versus-malignancy effect in the face of a low incidence of severe graft-versus-host disease in cord blood grafting will shed light on this "holy grail" of allogeneic stem cell transplantation. The field of cord blood transplantation holds promise for many patients who have few other options, but its success is critically dependent on a broad donor pool particularly for ethnic minorities that are under-represented in traditional bone marrow registries.
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**Cord blood for allogeneic and autologous banking**

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Introduction

The first successful allogeneic cord blood (CB) stem cell transplantation has been performed in 1988 in a boy with Fanconi’s anemia using the HLA-identical hematopoietic stem cells from the cord blood of his newborn sister (Gluckman E et al. NEJM 1989). Today this boy is healthy with a functioning donated hematopoietic and lymphatic system. Since then, the collection, storage and transplantation of stem cells derived from cord blood has become a widely used alternative method to treat patients with malignant and non-malignant hematological diseases, congenital immunodeficiencies and solid tumors (Gluckman E, Rocha V. Sem Immunopath 2004). More than 3500 allogeneic cord blood transplantations have been performed worldwide. Cord blood represents nowadays a significant source of stem cells, not only for pediatric patients (approximately 15% of transplants are derived from cord blood), but also in adults (http://www.netcord.org; http://www.marrow.org). Using improved supportive therapy to circumvent the limitations of the restricted number of stem cells derived from cord blood, adults now account for about one third of all CB recipients. Most of the transplantations performed were allogeneic, only few autologous. However, in contrast to the established public CB banks, commercial private banks focus particularly on these autologous transplantations. This policy is a matter of debate, because it is not clear yet, what future potentials for instance primitive stem cells derived from cord blood have in terms of their use in regenerating tissue.

Advantages and disadvantages of HSC from cord blood
Approximately 30 percent of patients in need for hematopoietic stem cell (HSC) transplantation will have a matched related donor and in 50 to 80 percent an unrelated suitable matched donor will be identified (Sanz MA, NEJM 2004). However, two months time which is required to identify the matched donor and obtain the graft might be too long in some cases where an urgent transplantation is indicated due to the progression of disease. If a suitable HLA-matched donor is missing and the application of an allogeneic bone marrow transplant is further limited by the increasing incidence of graft versus-host disease (GvHD) with increasing HLA disparity, the use of alternative sources of HSC, such as cord blood-derived stem cells, is an important advance in this field. A cord blood bank with already HLA-typed transplants stored frozen and ready to use represents an excellent alternative (Broxmeyer H et al. Proc Natl Acad Sci 1989). If one would be able to obtain HSC from cord blood of nearly all newborn infants, also very rare HLA types would be present in the CB bank and available for the population (see table 1). Besides the relatively short time between request of a HSC transplant and actual transplantation of stem cells from cord blood (Barker JN et al. Biol Blood Bone Marrow Transplant 2002), HSC from CB have other important advantages: the risk of virus transmission (CMV, EBV etc.) is lower than in grafts obtained from adult donors. The cord blood is collected from the already clamped part of the umbilical cord and placenta without risk and pain to the donor if the natural course of labor including the timing of cord clamping is not affected by the later CB collection procedure. The risk of severe graft versus-host disease is decreased because the co-transplanted leucocytes are immunological immature (Rocha V et al. NEJM 2000). This immunological incompetence leads on the other hand to a decreased graft versus-leukemia (GVL) effect which would be relevant in the treatment of malignant diseases. One major drawback of stem cells derived from cord blood is their limited number: on average 50 – 200 ml CB are collected with a certain number of nucleated cells (Surbek DV, Schönfeld B et al. Bone Marrow Transplant 1998). If the transplant is derived from an allogeneic anonymous donor, any booster transplantation to enhance the engraftment level is excluded. For a successful engraftment however at least $1 - 2 \times 10^7$ nucleated cells/kg are needed, thus HSC transplantation is mainly restricted to infants and is available for adults only under certain circumstances, e.g. low body weight, or in the absence of other
Several strategies are now investigated to deal with these limitations. It has been shown that the growth potential of HSC derived from cord blood is significantly elevated when compared to HSC from adult sources (Gluckman E et al. NEJM 1997, Lu L et al. Blood 1993). It seems that HSC can be expanded in-vitro (Shpall EJ et al. Biol Blood Bone Marrow Transplant 2002). However, it remains unclear whether early progenitors are lost during this in-vitro treatment and thus the long term repopulation capacities would be negatively affected. Other strategies to increase the engraftment potential are the co-transplantation of HSC with stromal cells (mesenchymal stem cells; MSC) and tandem transplantations of two CB grafts (Almeida-Porada G et al. Blood 2000, Zou HY et al. Zhongguo Shi Yan Xue Za Zhi 2004). Interestingly, the transplantation of two allogeneic grafts leads to a long term engraftment of only one of these. A co-transplantation of third-party MSC alleviates single-donor predominance and increases engraftment of HSC from CB in a NOD/SCID mouse model (Kim D-W et al. Blood 2004).

Another disadvantage which is related to the early time point of stem cell collection in donor’s life is the fact, that certain genetic diseases are not obvious at delivery, thus any transmission of genetic diseases through the CB graft can not be completely excluded. The risk can be minimized by the application of very strict exclusion criteria in the donor selection process and optimally by follow up examinations of the donors before the CB transplant is actually released from the CB bank.

Factors that influence the number of HSC from cord blood

The survival after transplantation of HSC from cord blood is similar or even better than after HSCT from bone marrow. However, it has been shown that the number of cells is critical for the time to reconstitute recipient’s bone marrow and thus is directly correlated to transplant related complications (Rocha V et al. Blood 2001). For the characterization of hematopoietic potentials of the transplant the number of nucleated cells is currently used. To improve the estimations the CD34+ cells and colony-forming units (CFU) are determined (Migliaccio AR, Adamson JW, Stevens CE et al. Blood 2000).

In cord blood 1 – 3 % of nucleated cells are hematopoietic stem cells (Knudtzon S Blood 1974). When compared to adult peripheral blood, in cord blood the number of CD34+ and
CD34+CD38- cells is 10fold increased, thus comparable to bone marrow (Broxmeyer H et al. Proc Natl Acad Sci 1989, Hao QL et al. Blood 1995). This elevation is due to a transfer of hematopoietic sites from liver and spleen to fetal bone marrow in the third trimester (Surbek DV, Holzgreve W et al. Am J Obstet Gynecol 1998). Thus the numbers of CD34+ and CD34+CD38- are significantly elevated in the 2nd and early 3rd trimester compared to term (Wysz A et al. Exp Hematol 1999). In consequence, any CB collection after preterm delivery would lead to less CB volume and number of nucleated cells, but could still provide sufficient numbers of HSC. Intrauterine and intrapartal stress due to preeclampsia, IUGR, vaginal operative delivery, cesarean section, prolonged labor, low umbilical arterial pH would also increase the amount of HSC in fetal peripheral blood probably due to increased release from the hematopoietic sites (Ballen KK et al. Bone Marrow Transplant 2001, Aufderhaar U et al. J Perinat Med 2003, Donaldson C et al. Br J Haematology 1999). A positive correlation between CD34+ cells and birth as well as placental weight has been reported. The timing of CB collection is also an important factor for the recovery of HSC from CB: collection before the expulsion of the placenta would increase the number of HSC. A very early cord clamping and CB collection would increase the recovery. However, it is an ethical issue if delivery of the infant would be affected by the later collection procedure and is generally not recommended (Yao AC et al. Lancet 1969, Surbek DV, Visca E et al. Am J Obstet Gynecol 2000, Nakagawa R et al. Transfusion 2004).

Clinical outcome of allogeneic cord blood HSC transplantation

The first allogeneic successful cord blood HSC transplantation in 1988 opened the way for a new field. Since then, knowledge of the biological characteristics of umbilical cord blood-derived stem cells has increased. Accordingly, umbilical cord blood banks have been established for allogeneic related and unrelated HSC transplantation (Rubinstein P et al. Blood 1993). Patients with a manifest malignant disease in need for a rapid HSC transplantation are those who benefit most from these established HSC banks with frozen, already HLA-typed and ready-to-use transplants. In contrast to unrelated bone marrow transplants in which a complete HLA identity is required, most of the cord blood HSC transplantations have been performed with donors having one to three HLA mismatches (see table 2). It is known from many studies that outcome after HSC
transplantation depends on the number of cells in the transplant (total nucleated cells TNC, CD34+ cells, CFU) and on the number of HLA mismatches (Gluckman E Exp Hematol 2000).

The number of nucleated cells infused should be optimally greater than 3 \times 10^7/kg (Gluckman E et al. NEJM 1997). It has been shown however, that outcome, as defined here by the time required to reconstitute 500 neutrophils per µl (ANC500), is more significantly correlated with the number of CFU than TNC (Migliaccio AR et al. Blood 2000).

Outcome of umbilical cord HSC transplantation in children

It has been shown that transplant-related mortality is related to cell count. After transplantation of about 10^7 NC per kg mortality was 75%, whereas transplant-related mortality decreased to 30% after injection of 3 \times 10^7 NC per kg (Gluckman E et al NEJM 1997).

Compared to the transplantation of related allogeneic HSC the time to reconstitute recipient’s hematopoiesis lasts longer, however, long term survival rates are similar. As stated above, in contrast to bone marrow transplantations, HLA mismatches can be accepted. In cases with hematological malignancies in children HLA mismatches represent a good therapeutic option to obtain complete remission (Ohnuma K et al. Br J Haematol 2001). Besides major histocompatibility antigens, it seems that also minor histocompatibility antigens play a significant role in the graft versus-host resp. graft versus-leukemia effects of transplants. When umbilical cord blood HSCT is compared with not manipulated BMT in children, it has been shown that although CB-derived HSC shown a higher HLA mismatch, the engraftment probability at day 45 (88 vs. 96%; p=0.41), the risk of chronic GvHD (53 vs. 41%; p=0.40) and 2-year event-free survival (53 vs. 41%; p=0.40) are similar (Barker IN et al. Biol Blood Bone Marrow Transplant 2002). In contrast to these long term results, CBT is related to increased short term mortality (day 100: 39 vs. 19%) because the reconstitution of hematopoietic lasts significantly longer (ANC500: 29 days vs. 22 days) (Gluckman E et al. Transfus Clin Biol 2001).

In contrast to allogeneic unrelated CBT, the reconstitution of hematopoiesis after related CBT lasts longer. The incidence of HLA matched transplants is significantly higher in related CBT (75-90 vs. 10%) (Gluckman E et al. NEJM 1997, Wagner JE, Kurtzberg J Curr Opin Hematol 1997, Wagner JE et al. Lancet 1995). Better engraftment is found in children of younger age and lower
Similar to related CBT the risk of GvHD is lower in transplants with better HLA match (9 vs. 50%) (Gluckman E et al. NEJM 1997). When compared to unrelated transplantsations (bone marrow and cord blood), related CBT results in similar long term transplant-related mortality, overall survival and 3-year survival rate (Rocha V et al. NEJM 2000). Not only in malignant diseases, but also in the treatment of thalassemia and sickle cell anemia promising survival rates can be achieved (2-year survival rate: 79-90%) (Locatelli F et al. Blood 2003).

In summary, allogeneic related and unrelated CB are very good alternatives to the classical stem cell sources such as bone marrow. The obstetrician in charge of the pregnant women, who reports on a sibling requiring a HSC transplantation, should be aware of this therapeutic option. On the other hand, a selection of an embryo based on its HLA-type during the IVF procedure aimed to function as a HSC donor at delivery, is ethically very problematic. Here, the sibling would be reduced to a stem cell donor.

Outcome of umbilical cord HSC transplantation in adults
Although at the beginning of the development the application of cord blood-derived HSC transplantation was restricted to children due to the limited number of transplanted cells, it has been shown recently that in adults transplantations using this source is a promising therapeutic option (Rubinstein P et al. NEJM 1989). The first large multicenter study by Laughlin and co-workers reported on unrelated CBT in 68 adults where no related or unrelated bone marrow donor was available. In most of these patients CB grafts with two or three HLA-mismatches were used. After injection of $2.1 \times 10^7$ NC/kg 26% of patients were disease-free at 22 months. The study showed that when compared to BMT, CBT resulted in slow myeloid engraftment (Neutrophils in 90% of patients at day 27), less problematic acute and chronic GvHD (acute: 60% of patients; chronic: 38% of patients) and increased early mortality (57% in 3 months) (Laughlin MJ et al. NEJM 2003). The biggest trial was performed by Gluckman and associates on 108 patients who received CBT because of a malignant disease, on average $1.7 \times 10^7$ nucleated cells per kg were infused. The outcome was best, if more than $1.7 \times 10^7$ NC/kg were transplanted and if the patient did not suffer from CML (Gluckman E. NEJM 2001). In contrast to BMT, the duration of aplasia seems to be longer,
which is problematic in terms of transplant-related mortality, e.g. bacteriemia (Hamza NS et al. Br J Haematol 2004). The risk of acute and chronic GvHD seems to be higher in adults than in children, but still lower than after unrelated BMT, most probably due to the lower number of co-transplanted CD3+ lymphocytes and the immunological immaturity of lymphocytes in CB (Laughtin MJ et al. NEJM 2001, Han P et al. Br J Haematol 1995).

In summary, CBT is a feasible option in adult patients when a sufficiently matched classical bone marrow graft is missing. However, the limited cell number in CB grafts is critical and several attempts have been made to improve the outcome results in adults. One promising approach is to transplant more than 1 CB. Barker and co-workers reported on 23 tandem transplantations with a combined cell dose of at least $2 \times 10^7$ NC/kg (Barker JN et al. Blood 2005). There was no graft failure; full donor chimerism was established for one of the two transplanted grafts at day 100. The predominating CB was not predictable by TNC, CD34+, CFU, HLA match, locus of HLA mismatch, donor sex, AB0 type nor sequence of injection. Compared to single CBT, tandem transplantation have an at least similar rate of engraftment failure and transplant-related mortality, rate of GvHD and an equal or higher disease-free survival at 1 year. Several other approaches to improve outcome by shortening the engraftment times and/or increase engraftment levels include ex vivo expansion of hematopoietic progenitors from CB, coinfusion of large doses of haploidentical CD34+ cells from related donors, co-transplantation of mesenchymal stem cells and ex vivo cell culture to improve stem cell homing (Jaroscak J et al. Blood 2003, Fernandez MN et al. Exp Hematol 2003, Almeida-Porada G et al. 2000). However, it is not clear yet, whether primitive progenitors are lost during the in vitro procedure and thus long term engraftment properties would be negatively affected. It seems that the engraftment kinetics is not increased by in vitro expansion (Sphall et al. Biol Blood and Bone Marrow Transplant 2002). The optimization of cytokines and the addition of MSC to increase the number of CD34+lin-cells is currently under investigation (Laens KM et al. Blood 1998, Pacchella W et al. Blood 1999). Since HSC and MSC might be sequestrated in the liver and lung after iv-injection, another attempt was made to increase engraftment by direct intra-bone marrow injection (Castello S et al. Exp Haematol 2004).
Controversies in allogeneic and autologous CB banking

Cord blood stem cells are increasingly used to repopulate bone marrow in the treatment of malignant and non-malignant diseases in children and adults. This development, however, is also related to a continuing debate on the role of public versus private cord blood banks.

Public cord blood banks store HSC for allogeneic, usually unrelated, transplantations. Currently between 175,000 and 200,000 units are stored frozen worldwide (Steinbrook R. NEJM 2004). The infant and its parents donate the CB to the bank and therewith to the public. Unlike private CB banks, public banks do not charge for collection and storage. Most of the transplants are used for the allogeneic treatment of leukemia, about a quarter for the treatment of genetic diseases. Similarly, a related allogeneic HSC transplantation using the stored CB from a sibling is well established in public banks. Public CB banks have the opportunity to provide HSC also for ethnic minorities that are underrepresented in bone marrow registries.

Private cord blood banks market their service to the expectant parents. They store CB for future use of the donor or its family and charge between $1,000 and $1,500 for collection and $100 per year for storage (Med Lett Drugs Ther 2004). Currently, CB from private banks is unlikely to be ever used. The probability of needing an autologous transplant is less than one in 20,000 (Annas GJ. NEJM 1999). Best estimates suggest the risk at 1 in 2,700 (Johnson FL. J Pediatr Hematol Oncol 1997). In some cases, private banked CB has already been used for a sick sibling; the probability of HLA match is 25%. The most likely indication for an autologous HSC transplantation is an acute leukemia. However, in this case, there are arguments against an autologous CB transplant, since already at the time of CB collection the predisposing mutation might be present. The rate of relapse after an autologous HSCT is higher (Burgio GR et al. Lancet 2003). Other current indications of autologous HSC transplantation are solid tumors, lymphomas and auto-immune diseases. But here again the probability of ever using the collected CB is very low, and often the number of collected cells is not sufficient for adults. In this case, it would be possible to harvest HSC from other autologous sources such as bone marrow or peripheral blood. Based on
today’s knowledge, several scientific and public societies, such as the American Academy of Pediatrics, American College of Obstetrics and Gynecology, Royal College of Obstetricians and Gynaecologists, French national Consultative Ethics Committee for Health and Life Sciences and the European Union do not recommend commercial CB banking (American Academy of Pediatrics Work Group on Cord Blood Banking. Pediatrics 1999, ACOG Committee Opinion. Int J Gynaecol Obstet 1997, RCOG Scientific Advisory Committee. http://www.rcog.org.uk/mainpages.asp?PageID=430, French National Consultative Ethics Committee for Health and Life Sciences. http://www.ccne-ethique.fr/english/start, European Union on ethics in Science and New Technologies. http://europa.eu.int/comm/european_group_ethics/docs/avis19_en.pdf). Major critics arise from the question on the cost-to-benefit ratio of private banking in a low risk population. However, it is already known that CB contains several types of progenitors, hematopoietic and mesenchymal stem cells (Prockop DJ. Science 1997). Recently, more immature progenitors with multilineage potential have been isolated from CB. Though low in number, it is difficult to speculate about the potentials of these cells (Kögler G et al. J Exp Med 2004). Besides, it has been shown that stem cells might exhibit a so-called “plasticity”, the quantity and functional relevance of these events is a matter of debate (Petersen BE et al. Science 1999, Krause DS et al. Cell 2001). Although these primary data on transdifferentiated cells were the result of cell fusion, more recent data show the isolation of multipotent progenitor cells from cord blood that have the potential to differentiate into cells of all three cell layers, mesoderm, endoderm and ectoderm (Wang X et al. Nature 2003, Jiang Y et al. Nature 2002, Kögler G et al. J Exp Med 2004). Thus, it seems that stem cells derived from cord blood and probably expanded and treated in vitro have the potential to be used for tissue regeneration in common diseases such as heart failure, Parkinson’s disease or Diabetes mellitus. It needs to be discussed who should cover the costs of stem cell collection and such regenerative treatment in the future. At the moment, parents with low income are excluded from this type of stem cell banking.

It is therefore important to discuss the financial aspect of autologous stem cell banking in the public resp. ethics committees and let the commercial CB banks correctly advertise about the probabilities of ever needing an autologous cord blood stem cell transplantation for current indications and the – though experimental – potentials of stem cells derived from CB.
Table 1  Advantages and disadvantages of grafts derived from bone marrow compared to cord blood (modified from Gluckman et al. Seminars in Immunopathol 2004)

<table>
<thead>
<tr>
<th></th>
<th>Bone marrow</th>
<th>Cord blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median search time</td>
<td>3-6 months</td>
<td>&lt; 1 month</td>
</tr>
<tr>
<td>Donors identified but not available</td>
<td>30%</td>
<td>&lt; 1%</td>
</tr>
<tr>
<td>Rare HLA types represented</td>
<td>2%</td>
<td>29%</td>
</tr>
<tr>
<td>Major limiting factors to graft acquisition</td>
<td>HLA match</td>
<td>Cell dose and HLA</td>
</tr>
<tr>
<td>Potential for second (booster) HSC graft or donor lymphocyte infusion</td>
<td>Yes</td>
<td>Not from the same donor</td>
</tr>
<tr>
<td>Potential for viral transmission</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Potential for congenital diseases</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Risk for donor</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

Table 2  Patient and transplant-related characteristics of 1083 CBT reported to Eurocord (modified from Gluckman E, Rocha V. Springer Seminars Immunopathol 2004)

<table>
<thead>
<tr>
<th></th>
<th>Related CBT n=206</th>
<th>Unrelated CBT n=877</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Children (&lt;15 years)</td>
<td>200 (97 %)</td>
<td>624 (71.2%)</td>
</tr>
<tr>
<td>Adults</td>
<td>6 (3%)</td>
<td>253 (28.8%)</td>
</tr>
<tr>
<td>Indication</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignancies</td>
<td>100 (48.4%)</td>
<td>691 (78.8%)</td>
</tr>
<tr>
<td>Bone Marrow failure syndromes</td>
<td>34 (16.5%)</td>
<td>69 (7.9%)</td>
</tr>
<tr>
<td>Inborn errors</td>
<td>18 (8.8%)</td>
<td>115 (13.1%)</td>
</tr>
<tr>
<td>Hemoglobinopathies</td>
<td>53 (25.7%)</td>
<td>0</td>
</tr>
<tr>
<td>Others</td>
<td>1 (0.5%)</td>
<td>2 (0.2%)</td>
</tr>
<tr>
<td>HLA disparities</td>
<td>Matched</td>
<td>1</td>
</tr>
<tr>
<td>-----------------</td>
<td>---------</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td>187 (90.7%)</td>
<td>5 (2.4%)</td>
</tr>
<tr>
<td></td>
<td>89 (10.1%)</td>
<td>374 (42.6%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Transplant-related factors</th>
<th>Nucleated cells infused (10^7/kg)</th>
<th>CD34+ cells infused (10^7/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4.0 (0.7-80.8)</td>
<td>1.8 (0.06-63.0)</td>
</tr>
<tr>
<td></td>
<td>3.6 (0.1-94.6)</td>
<td>1.6 (0.01-78.0)</td>
</tr>
</tbody>
</table>

References


34. Luens KM, Travis MA, Chen BP et al. (1998) Thrombopoietin, kit ligand, and flk2/flt3 ligand together induce increased numbers of primitive hematopoietic progenitors from human CD34+Thy-1+Lin- cells with preserved ability to engraft SCID-hu bone. Blood 91: 1206-1215.


Ex Vivo Expansion of Cord Blood

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Umbilical cord blood (CB) provides an alternate source for patients undergoing high dose chemotherapy for treatment of cancer or genetic diseases. In particular, CB has become a standard therapeutic option for selected patients with hematologic

malignancies. Several studies have reported on the use of CB for transplantation in adult patients, however, the low cell doses has limited the use of CB in this setting due to subsequent delays in engraftment. Ex vivo expansion is one approach that is being explored as a means to provide larger cell numbers from CB products.

**Cellular Content of CB:**

CB products contain similar cell populations to bone marrow (BM) and mobilized peripheral blood progenitor cell products (PBPC), including hematopoietic stem cells (HSC), primitive progenitor cells, mature progenitor cells and mature functional cells. However, the total cell number and progenitor cells are much lower in CB compared to BM and PBPC. For example, BM and PBPC contain approx $10^8$ CD34+ cells while CB contains approximately $5 \times 10^6$ CD34+ cells. In contrast the frequency of HSC, as determined by NOD/SCID engraftment is enriched in the CD34+ cell population of CB compared to BM or PBPC. As few as 100,000 CB CD34+ cells can engraft NOD/SCID mice, while approx 1 million BM CD34+ cells and 5 million PBPC CD34+ cells are required for engraftment of human cells.

These numbers would suggest that CB contains similar levels of HSC to BM and PBPC but significantly lower levels of committed progenitor cells. The use of these cellular grafts in the clinical setting, result in data that support this theory. Patients transplanted with CB grafts have delayed neutrophil and platelet engraftment compared to patients transplanted with BM or PBPC products, however there does not appear to be any long term engraftment problems in patients transplanted with CB grafts. This suggests that CB products contain sufficient long term engrafting cells (HSC), but minimal short term engrafting cells (mature progenitor cells).
Therefore a simple goal of ex vivo expansion would be to generate more committed progenitor cells that have the potential to provide faster short term engraftment. This can be achieved by ex vivo culture in hematopoietic growth factors (HGFs), however we must consider the potential negative impact of depleting HSC by driving their differentiation to mature progenitor cells. Therefore the ideal protocol for evaluating ex vivo expanded CB cells involves the use of two products, one component which has been ex vivo expanded and the second that has not been manipulated.

**Ex Vivo Expanded Cells Provide Rapid Engraftment:**

The potential enhancement of engraftment by ex vivo expanded cells has been demonstrated in clinical trials. Several studies [1-4] have been reported using ex vivo expanded PBPC CD34+ cells in myeloablated patients. In these studies the use of ex vivo expanded cells resulted in faster neutrophil engraftment, with patients having minimal days of neutropenia compared to patients receiving unexpanded PBPC products. Our own study conducted at the University of Colorado [4], resulted in neutrophil engraftment as early as 4 days post transplant. Analysis of the patient data demonstrated minimal correlation of the time to engraftment to CD34+ cell dose, but demonstrated a highly significant correlation to the dose of total nucleated cells per kg of body weight of the recipient [4]. Evaluation of cytospins prepared of the expanded cells demonstrated a high percentage of mature neutrophil cells. Based upon these data we have focused our experimental protocols on driving differentiation of CB cells to produce a cellular product that contains a high proportion of mature neutrophil cells. In addition, these conditions drive the production of mature progenitor cells [4,5]. These conditions also appear to deplete products of long term engrafting cells as demonstrated by
engraftment of fetal sheep [6]. Again indicating the need for developing clinical protocols that utilize two graft components, one expanded and the second unmanipulated.

**Selection of CB Products for Ex Vivo Expansion:**

A number of systems have been explored for ex vivo expansion of CB products from liquid culture in bags to bioreactors. A number of groups have demonstrated that selection of CD34+ cells or CD133+ cells is necessary for optimal expansion. In 1997 we reported that culture of CB mono nuclear cells (MNC) in a HGF cocktail of stem cell factor (SCF) plus granulocyte colony stimulating factor (G-CSF) and thrombopoietin (Tpo) resulted in only a 1.4 fold expansion of total cells, 0.8 fold in mature progenitor cells (GM-CFC) and 0.3 fold in erythroid progenitors (BFU-E) [7]. In contrast, CD34+ selected CB cells resulted in 113 fold expansion of total cells, 73 fold expansion of GM-CFC and 49 fold expansion of BFU-E. Based upon these results we have initiated expansion cultures in clinical trials with CD34-selected CB cells. Processing of clinical products has led us to two conclusions:

1) Although we can expand significantly the TNC and committed progenitor cells from CD34+ cells we rarely reach pre selection total cell numbers. For a typical CB product starting with a cell dose of $1 \times 10^9$ TNC and containing 0.5% CD34+ cells, we would obtain a maximum of $5 \times 10^6$ CD34+ cells post selection. Therefore after culture for 10 to 14 days we would require a minimum of 200 fold expansion of TNC to obtain pre processing levels.

2) The performance of clinical trials using CB grafts in the unrelated setting requires the use of frozen CB products. Selection of frozen CB products results in significant losses of CD34+ cells (50% or greater loss of CD34+ cells) and often results in low purities [8]. With a 50% recovery of CD34+ cells after selection we now require at least a 400 fold
cell expansion to obtain equivalent TNC as we started with. Again in our experience with clinical studies the purity of the CD34-selected product impacted the levels of expansion achieved. The median purity of CD34+ cells was 47.5% (range 14 to 81%) and the median expansion was 56 fold of TNC. Products with a purity greater than 50% resulted in a median of 139-fold, while products with a purity less than 50% resulted in only 32-fold expansion.

Therefore in our experience to date the use of CD34 selected products has rarely resulted in increased cell doses of ex vivo expanded cells compared to the starting unmanipulated product.

**Availability of Clinical Grade Reagents:**

A number of approaches have been evaluated for ex vivo expansion of CB products including various culture media, HGF cocktails and various culture vessels (flasks, bags etc). Most protocols utilize a 10 to 21 day culture in 5%CO$_2$ incubators, so the development of closed culture systems using clinical grade HGFs and media is essential to comply with regulatory body requirements. In our culture system we have used three HGFs, namely, SCF, G-CSF and Tpo as these HGFs have been manufactured to GMP (Good Manufacturing Practices) standards. It is most likely that addition of other HGFs could enhance the expansion potential of these cultures, however the lack of GMP grade inhibits translation to clinical trials. Similarly media must be manufactured to GMP and there limited options available. In our initial clinical trials we used a defined media that was manufactured by Amgen for clinical use, however, Amgen discontinued the production of this media and in subsequent expansion trials we have been using Sigma’s Stemline II expansion media. This is a proprietary media with the formulation a trade secret to prevent duplication by other manufacturers. Sigma manufacture this
defined media to GMP grade and we have recently initiated two expansion clinical trials using it.

**Clinical Experience With Ex Vivo Expanded Cells:**

Despite hundreds of reports of preclinical studies evaluating ex vivo expansion of CB products only a small number of clinical trials have been conducted to evaluate the clinical potential of ex vivo expanded CB cells. Kurtzberg et al [9] (n=21 patients) and Stiff et al [10] (n= 9 patients) used the Aastrom system for expansion of CB cells however, no significant effects on engraftment kinetics were observed in these patients. We have reported the results of a clinical trial we conducted at the University of Colorado [11] and again the conclusion was that the rate of engraftment was not significantly increased by the use of expanded cells. Several ongoing trials at MD Anderson have been reported at meetings by Dr. Shpall [12] and again the data suggest that the ex vivo expanded cells have had minimal impact on the rate of engraftment. These studies suggest that the culture conditions currently being undertaken are not capable of expanding the appropriate cell population or that insufficient numbers are being generated to impact the time to recovery of neutrophils or platelets. Our conclusion from our own experience and data is that the requirement for selection of CD34+ cells or CD133+ cells from frozen CB products, greatly minimizes the potential of generating a suitable expanded CB product to enhance the rate of engraftment. Therefore, in recent studies we have evaluated methods for expanding CB products without an initial CD34- or CD133-selection.

**Ex Vivo Expansion of CB MNC on MSC:**
We have developed a co culture system which is capable of expanding CB MNC by culturing the CB MCN on confluent MSC layers. The literature contains many reports of the ability of MSC to support the growth of hematopoietic cells. It has been demonstrated that MSC produce a number of HGFs and adhesion molecules that may stimulate growth of hematopoietic cells [13]. Our initial data reproducibly demonstrated a 10 to 20 fold expansion of TNC with 18 fold expansion of GM-CFC and 16 to 37 fold expansion of CD34+ cells.

In recent experiments we have evaluated the potential of ex vivo expansion of frozen CB products using the co culture on MSC. CB products were thawed and washed resulting a median of $3.3 \times 10^8$ TNC (range 1.4 to 3.6 $\times 10^8$). For a 50 kg recipient, these CB products would provide only $0.73 \times 10^7$ TNC/kg with zero of 5 products reaching the minimal target dose of $1 \times 10^7$ TNC.

Each product was expanded by culturing the MNC fraction from each product on preformed layers of MSC. Ten T162 cm² flasks were used for each product such that each flask contained 10% of the CB MNC. After ex vivo culture for 14 days in the cocktail of SCF, G-CSF and Tpo in Stemline II media, a median of 9 fold expansion of TNC was obtained with a range of 6.5 to 24 fold. The median TNC post expansion was $21.6 \times 10^8$ (range 11 to 79 $\times 10^8$) (Table 1). A median expansion of mature progenitor cells (GM-CFC) of 46 fold was also obtained in the co culture. For a 50 kg recipient, the expanded CB product would be equivalent to $4.3 \times 10^7$ TNC/kg (range 2.2 to 16 $\times 10^7$), with all 5 expanded products reaching the minimal target of $1 \times 10^7$ TNC/kg. In fact all expanded products contained more the $1 \times 10^7$ TNC/kg based upon a 100 kg recipient.

We would propose two potential advantages to the use of co culture for expansion based upon these results. Firstly the possible enhanced engraftment and secondly the
ability to use better matched CB products that may have a low cell dose. Wagner and colleagues have been transplanting two CB products to provide an increased cell dose however, the majority of patients receive a 2 antigen miss matched CB unit [14]. Better matched CB units are routinely identified but are not suitable due to low cell doses. The expansion of these CB units would enable at least on unit to be better matched to the recipient and potentially decrease the graft versus host disease that can result.

A clinical trial to evaluate the potential of ex vivo expanded cells generated using this co culture approach, is currently being planned for conduct at MD Anderson and it is anticipated that this study will commence in the 3rd quarter of 2006.

Summary:
Despite more than a decade of research the clinical results for ex vivo expanded CB products have not provided a major impact on time to engraftment of the recipients. New approaches are currently being developed and it is hoped that these trials will demonstrate faster engraftment and provide a platform for optimization of the development of protocols for CB transplant. It is clear from the trials that have been undertaken to date, that we still have not clearly identified the cell population, required to provide rapid engraftment. We can only optimize culture of this cell population once we identify its phenotype. This highlights the need for continued clinical trials and development of large animal models that may shed light on what cells and how many are required to provide an optimal hematopoietic cell graft.

Disclosure Statement:
Under a licensing agreement between ViaCell Inc, and the Johns Hopkins University, Ian McNiece is entitled to a share of royalty received by the University on sales of products
described in this article. The terms of this arrangement are being managed by the Johns
Hopkins University in accordance with its conflict of interest policies.

REFERENCES


### Table 1: Ex Vivo Expansion of TNC from Frozen CB Products

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### Table 2: Cell Doses Based Upon Recipient Weight of 50 kg (A) and 100 kg (B)

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Double Umbilical Cord Blood Transplantation in Adults

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Key Words: Umbilical Cord Blood; Transplantation

Abstract:

The first successful cord blood transplant was reported in 1989 in a child with Fanconi’s anemia. Over the last seventeen years, there has been a dramatic growth in the use of cord blood as an alternative stem cell source for patients without matched related or unrelated bone marrow donors. Initially, the majority of transplants were performed in children. Recently, the results in adult cord blood transplantation appear promising. We will address in this chapter outcome data for adult cord blood transplantation, with an emphasis on new techniques using double or sequential cord blood transplantation. New indications for cord blood use outside of hematology/oncology will also be explored.
Preclinical Background:

Work in the laboratories of Broxmeyer, Knutson-Knudtzon, Lansford, Metcalf, and others showed that neonatal and cord blood contained a high number of granulocyte-macrophage progenitor cells. In a murine model, neonatal mice contained adequate progenitor cells for bone marrow reconstitution in irradiated mice. Human cord blood was then shown to have sufficient progenitor cells to provide consistent hematopoietic engraftment. These studies provided the scientific support for the initial trials with cord blood transplantation.

Further work, after some of the initial clinical studies, revealed the unique immunologic properties of umbilical cord blood. Cord blood CD34+ cells in culture increase in cell number every seven to ten days, several hundred fold greater than the increase in cultures of similar cells from adult bone marrow, thereby allowing a 10 fold lower CD34+ cell dose to be used for successful cord blood transplantation. Cord blood cells have greater proliferative capacity; longer telomere length has been proposed as a possible explanation.

The immunologic properties of cord blood differ from mature bone marrow or peripheral blood stem cells, which may contribute to the decreased graft versus host disease following cord blood transplantation, with preservation of a graft versus leukemia effect. Cord blood contains a high proportion of “naive” phenotype T cells that are CD45RA+/CD45RO-, CD62L+. The chemokine receptor CCR5, expressed by TH1 T cells, is less abundant among cord blood T cells than adult T cells. Cord blood T cell receptors when compared to adult blood T cell receptors, have a less complex repertoire. Cord blood cells express T cells with less clonal diversity than expressed by adult peripheral blood.

Cord Blood Unit Availability

Potential HLA matched cord blood units can be found for patients in computerized registries such as National Marrow Donor Program, Netcord, the New York Blood Center, and other international registries. Currently, Bone Marrow Donors Worldwide lists 232,000 available cord blood units from 35 different cord blood banks in 21 countries. Most of the units have molecular typing available for Class II HLA alleles, and about half of the units have Class I molecular typing available. In the United States, the National Marrow Donor Program currently lists 45,000 cord blood units. The search process and determination of match can be quite complex, especially when looking for more
than one cord blood donor. Searching for a cord blood donor is generally faster than for a bone marrow donor, as there is no living donor to find, contact, retest, and consent.

One of the goals of the cord blood program is to increase the number of donations from non-Caucasian donors, since non-Caucasian patients have a more difficult time locating matched donors through the National Marrow Donor Program (NMDP) and other international registries. In the early years of cord blood banking, we found no increase in cord blood donations from minority donors compared to donations from minority donors to bone marrow registries in the same United States geographic area. In fact, in three of the areas surveyed, California, Florida, and Massachusetts, the cord blood banks recruited a lower percentage of minorities than the corresponding bone marrow donor centers.

As visibility of the cord banking efforts grew, a more diverse donor population was recruited. The American Red Cross cord blood banks revealed a diverse donor population: 64% Caucasian, 16% African American, 12% Hispanic, 4% Asian, 1% Native American, and 3% other. An unexpected finding was a lower CD34+ counts for black donors in the Midwest, Northwest, and North Carolina collection sites. Since the average unit from black donors has a lower CD34+ count, an important prognostic factor, more units may need to be collected to list those with a suitable cell dose. The Cord Blood Transplantation Study (COBLT) study also showed lower CD34+/CD38- population in African American and Asian donors.

**Clinical Studies in Cord Blood Transplantation**

**Studies in Children—Related Cord Blood Transplants**

The first cord blood transplants were performed in children. Lessons learned from the pediatric experience have allowed us to successfully transplant adults. The experience with adult and pediatric cord blood transplantation has been recently reviewed.

Umbilical cord blood has been used successfully in related transplants for both malignant and non-malignant diseases. The Eurocord group reported on 102 children with acute leukemia receiving cord blood transplants; forty-two received a related donor transplant. Twelve of these patients received a mismatched graft; neutrophil engraftment was 84% and two-year event free survival was 41%. A nucleated cell dose >3.7 x 10^7/kg correlated with engraftment. For non-malignant diseases, there has been experience in patients with thalassemia and sickle cell disease. In the Eurocord study, the 100-day transplant related mortality was 0; four patients experienced grade 2 acute GVHD. One patient with sickle cell disease and seven patients with thalassemia did not have
sustained donor engraftment. The two-year probability of event-free survival was 79% for thalassemia, and 90% for sickle cell anemia. Cord blood, which is a naturally T-cell depleted product, has a low risk of graft versus host disease, and may be well suited to the treatment of non malignant diseases, as there is no advantage to graft vs host disease/graft vs leukemia.

Since randomized studies between cord blood and bone marrow would be difficult to accomplish, Rocha et al performed a retrospective case control study. One hundred and thirteen related cord blood recipients were compared with 2052 related bone marrow recipients. Transplant related mortality was similar between the two groups, although the incidence of GVHD was lower in the recipients of cord blood transplants.

Studies in Children—Unrelated Cord Blood Transplants

The first 25 unrelated cord blood transplants were reported in 1996 by the Duke Transplant Team. Thirty percent of these patients were non-Caucasian, and had searched for an unrelated marrow donor for at least six months. The cord blood unit and the patient were mismatched at 1-3 HLA loci. Twenty-three of twenty-five patients engrafted. Two patients had graft versus host disease, and the event-free survival was 48% with a median follow up of 12 months. These data suggested that engraftment could occur even with cord blood units that were mismatched at two loci and that the risk of severe graft versus host disease was low.

The University of Minnesota reported on 102 children, with malignant and non-malignant diseases, transplanted with unrelated umbilical cord blood. Although the incidence of graft versus host disease was low (severe acute GVHD was seen in 11% of patients and chronic GVHD in 10% of patients), the risk of leukemia relapse was also low, 17% for standard risk patients and 45% for high-risk patients, supporting a preservation of the graft vs leukemia effect. These data support a preservation of the graft vs leukemia effect, without clinical graft vs host disease.

Cord blood transplantation has also been shown to be effective in metabolic storage diseases. Twenty children with Hurler’s syndrome received conditioning
with busulfan, cyclophosphamide, and antithymocyte globulin followed by infusion of unrelated, 1, 2, or 3 antigen mismatched cord blood. With a median follow up of 905 days, 17 of 20 children are alive with complete donor chimerism and normal peripheral blood alpha-1-iduronidase activity. The low risk of GVHD with cord blood transplantation suggests a unique role for cord blood transplantation for children with non-malignant diseases.

The pediatric experience has provided guidance in developing a successful adult cord blood transplant program. The pediatric data indicate that engraftment can occur without a high risk of graft versus host disease, even if the patient and cord blood donor are mismatched at two antigens. Therefore, the potential donor pool is considerably increased from unrelated bone marrow or peripheral blood stem cells. The incidence of severe graft versus host disease is low, but a graft vs leukemia effect is preserved. The cell dose infused is consistently an important marker for improved engraftment and survival; engraftment, particularly platelet engraftment, is prolonged after cord blood transplantation.

**Studies in Adults—Single Cord Blood Transplants—Ablative Regimens:**

In the last five years, there has been increased interest in cord blood transplantation for adult patients. Only 30% of patients have a matched sibling donor, and cord blood offers an alternative stem cell source. The initial cord blood studies were performed using single cord blood units, and are outlined in Table 1. Laughlin et al analyzed a large American experience in sixty-eight
adults, median age 31 years, who received cord blood transplants. The incidence of neutrophil engraftment was 90%, but the median time to engraftment was 27 days, longer than in historical controls of unrelated bone marrow. A higher number of CD34+ cells in the cord blood units correlated with improved engraftment and survival. Transplant related mortality was high in this series; 47% of patients died prior to Day +100, with infection the leading cause of death. With a median follow up of 22 months, the disease-free survival was 26%.

Results in most other adult cord blood transplant studies using single cord blood units have been poor. In a study by Long and colleagues, 57 adult patients with high-risk disease underwent single cord blood transplantation. The median days to neutrophil and platelet engraftment were 26 and 84 respectively. The transplant related mortality at Day +100 was 50%, with the majority of deaths related to infection. Actuarial projected three-year survival was only 19%.

The Cord Blood Transplantation Study Group (COBLT) study reported on results of 34 adult patients with high-risk disease receiving cord blood transplantation. The median nucleated cell dose was low at 1.7 x 10^7 NC/kg; primary graft failure occurred in 28% of patients and there were only two long-term survivors.

Two reports of retrospective case control studies comparing unrelated bone marrow transplants to unrelated umbilical cord blood transplants in adults were reported in the New England Journal of Medicine in 2004. The International Bone Marrow Transplant Registry compared survival after unrelated umbilical cord blood transplants to survival after unrelated bone marrow transplants. One hundred and sixteen adults with leukemia receiving unrelated, one or two antigen mismatched cord blood transplants were compared with 367 adults receiving matched unrelated donor bone marrow transplants and 83 patients receiving 1 antigen mismatched bone marrow transplants. Deaths related to infection were highest in the cord blood recipients, but acute GVHD was more common in the mismatched bone marrow cohort. Three-year leukemia free survival was highest (33%) for patients receiving HLA matched unrelated bone marrow transplants, but comparable (23% for cord blood and 19% for 1 antigen mismatched bone marrow) for patients receiving the other two donor sources.

The Eurocord group reported results of a retrospective study comparing outcomes of 98 adults with acute leukemia receiving unrelated one or two antigen mismatched cord blood with 584 adults with acute leukemia receiving unrelated matched bone marrow transplants. Neutrophil recovery was delayed after cord blood transplant (26 days vs 19 days). Graft failure occurred in 20% of the cord blood recipients, and 7% of the bone marrow recipients. Although the incidence of acute GVHD was 26% after cord blood transplant, and 39% after unrelated bone marrow transplant, relapse rates were similar. Therefore, this data suggests that the graft vs. leukemia effect is preserved in cord blood.
recipients, even without a high rate of clinical GVHD. The two-year leukemia free survival was comparable in both groups, 33% for cord blood and 38% for unrelated bone marrow. This study suggests (in contrast to the American study above) that outcomes after cord blood transplant may be similar to outcomes after matched unrelated bone marrow transplant.

The results of the American and European studies indicate a survival of 20-35% after unrelated single cord blood transplantation in adults. In contrast, the Japanese group has reported excellent outcomes after single adult cord blood transplantation. The outcomes of 68 adult unrelated cord blood recipients were compared with 45 adult unrelated bone marrow recipients. The 100-day transplant related mortality was a remarkably low 9% for cord blood recipients and 29% for unrelated bone marrow recipients. The two-year probability of disease free survival was 74% for cord blood and 44% for unrelated bone marrow. These results are superior to those reported in the American and European series, perhaps due to the smaller size and genetic homogeneity of this population. Therefore, in Japan, unrelated cord blood is often selected as the preferred stem cell source, even when a matched unrelated donor is available.

Adult Cord Blood Transplantation—Single Cord Blood—Reduced Intensity Regimen

Nonmyeloablative or reduced intensity transplant regimens have been used extensively over the last five years, to reduced transplant related mortality in patients who are older or who have co morbid features (Table 2). The use of this approach is particularly important given the average age of patients at diagnosis of leukemia and lymphoma is 60 years old. The Japanese group treated twenty patients with refractory lymphoma with a reduced intensity regimen of low dose total body radiation, fludarabine, and melphalan, followed by unrelated cord blood transplantation. Transplant related mortality continued to be similar to a myeloablative regimen, at 41% in this high-risk group of patients. Estimated progression-free survival was 50%. Thirty patients with refractory leukemias received a similar approach. The transplanted related mortality, even with a reduced intensity approach, was high at 27% and one year overall survival was 33%. The graft failure rate after reduced intensity cord blood transplantation was estimated to be 7% in one retrospective review of 123 patients.

The Duke group used a reduced intensity approach, with a conditioning regimen of fludarabine, cyclophosphamide, and horse antithymocyte globulin, in 13 refractory hematologic malignancy patients receiving single cord blood units. There was only one treatment related death prior to 100 days. The one-year event free survival was 43%.
These studies show that engraftment is delayed and transplant related mortality is high in adult patients, receiving single cord blood transplants. Most of the deaths, using either a myeloablative or reduced intensity approach, have been due to infection. The cell dose (either nucleated cell/kg or CD34+ cells/kg) correlated with outcome in most studies. Therefore, strategies to improve outcomes have included increasing the cell dose administered by sequential or double cord blood transplantation. Cord blood expansion is another option to improve engraftment and is discussed elsewhere in this book.

**Adult Cord Blood Transplantation— Double Cord Blood Transplantation— Myeloablative Regimen**

Sequential or double cord blood transplant describes the transplantation of two or more partially matched cord blood units, given to the same patient several hours apart. Thus, the cord blood units are “pooled” in the patient’s body, “in vivo.” Although immunologic rejection might be a concern with this approach, an early case report of patients infused with multiple, mismatched units suggested that crossed immunologic rejection would not occur.

The University of Minnesota transplant program tested the two cord blood unit approach, in both the ablative and reduced intensity setting. Barker and colleagues treated 23 adults with hematologic malignancies with an ablative conditioning regimen of cytoxan, total body irradiation, and fludarabine. Each patient received two cord blood units, 4/6 HLA match or better with each other and with the patient. The combined median nucleated cell dose was $3.5 \times 10^7$ NC/kg. All twenty-one evaluable patients engrafted, with a median days to ANC >500 of 23. Initial chimerism studies revealed the presence of both cord blood units, with one unit predominating by Day +100.

Aplastic anemia has also been successfully treated with cord blood transplantation. In a Chinese study, six patients with severe aplastic anemia were conditioned with cyclophosphamide and antilymphocyte globulin. Three patients received one cord blood unit and three patients received two cord blood units. Two patients died of infection; five patients had stable mixed chimerism, and four of six are alive and disease-free with a median follow-up of 20 months.
Adult Double Cord Blood Transplantation—Reduced Intensity Regimen

The results of double cord blood transplantation using a reduced intensity regimen are outlined in Table 4. Barker and colleagues transplanted either one or two cord blood units to achieve a minimum cell dose of $3.5 \times 10^7$ nucleated cells/kg. Patients whose cord blood unit did not meet this cell dose criteria received the double cord blood transplant. Cord blood units were a 4/6 HLA match or better with the patients and with each other. Twenty-one patients received conditioning with busulfan/fludarabine/low dose TBI and 22 patients (the second cohort) received cyclophosphamide/fludarabine/low dose TBI. Cyclosporine and mycophenolate mofetil were used for GVHD prophylaxis. Twenty-four of the 43 patients received two cord blood units. The first regimen with busulfan had an incidence of neutrophil engraftment of 76%, with the median days to an ANC of 500 of 26 days. One hundred day transplant related mortality was 48%. The second regimen with cyclophosphamide had an incidence of neutrophil engraftment of 94% with the median days to an ANC of 500 of 10 days. One hundred day transplant related mortality was 28%. Overall survival at one year was 39%. Interestingly, for patients receiving a reduced intensity regimen, the Day +10 myeloid recovery was autologous, and engraftment of the cord units occurred later after transplant. Extensive chimerism data was not reported with this study.

This group recently updated their data in abstract form, to include 95 patients. Seventy-eight patients received a double cord blood transplant and seventeen patients received a single cord blood transplant. The conditioning regimen was cyclophosphamide, fludarabine, and low dose total body radiation. Median cell dose was $3.6 \times 10^7$ and 94% of the units were either a 4/6 or 5/6 match with the recipient and with each other. Median time to neutrophil engraftment was 12 days with a 6% rate of primary graft failure. Incidence of grade III-IV acute GVHD and chronic GVHD were both 25%. Overall survival was 52% at one year and 44% at two years.
The Minnesota group has compared, in a retrospective series, outcomes after double and single cord blood transplantation. This study compared 29 patients with acute leukemia who received double cord blood transplants with 14 patients who received single cord blood transplants. All patients received the conditioning regimen of cyclophosphamide, fludarabine, and total body radiation and immunosuppression with CSA and mycophenolate mofetil (MMF). The minimal TNC dose was $2.5 \times 10^7$. The risk of relapse was less (54% vs 11%) for patients in complete remission (CR) 1 or CR 2 who received double cord blood transplants. There was no difference for patients with more advanced disease (CR 3 or relapse). This study suggests that there could be additional benefit in graft vs leukemia effect to the double cord blood approach, but the numbers of patients are small in this series.

An additional study from the same group compared outcomes of double umbilical cord blood transplantation (n=9) or HLA matched sibling transplantation (n=12) using a reduced intensity regimen in patients with advanced Hodgkin lymphoma. The incidence of acute and chronic GVHD, transplant related mortality, and progression-free survival were similar between these two groups. The two-year progression free survival rates were 25% for cord blood recipients and 20% for patients receiving matched sibling donor grafts.

We have pursued the sequential or double cord blood transplant approach in a Phase I study at the Massachusetts General Hospital and Dana Farber Cancer Institute. Patients received a reduced intensity conditioning regimen of fludarabine, melphalan, and rabbit antithymocyte globulin. Two cord blood units were infused on the same day; the cord blood units were an HLA 4/6 (A, B, DR) match with each other and with the patient, and achieved a combined cell dose pre-freeze of $>3.7 \times 10^7$ NC/kg. Twenty-one patients were treated with this approach. The median days to neutrophil engraftment were 20 days, and the median days to platelet engraftment (platelet count $>20 \times 10^9$/L unsupported were 41 days. There were three deaths prior to Day +100, one related to a CNS bleed, one related to sepsis, and one related to an EBV lymphoproliferative disorder. There were three deaths after Day +100, one related to chronic GVHD and sepsis, one related to fungus infection, and one related to an EBV lymphoproliferative disorder. The one-year overall survival was 71% and the one-year disease free survival was 67%.
Adult Cord Blood Transplantation—
Chimerism Analysis

Chimerism refers to the amount each cord blood or the recipient contributes to hematopoiesis. Cord blood, particularly double cord blood transplantation, offers unique challenges in the technical aspects and interpretation of post transplant chimerism. Chimerism assays are usually performed by DNA methods using amplification of short tandem repeat loci by polymerase chain reaction. The published studies of double cord blood transplantation have shown, that typically one cord blood units predominates and provides the majority of the hematopoiesis.

In the study by Barker et al using a myeloablative conditioning regimen, 76% of the patients showed hematopoiesis from only a single cord by day 21. By Day +100, one cord unit predominated in all patients. Chimerism results have been analyzed in detail for our study of 21 patients treated with a reduced intensity regimen.

Ten of 18 evaluable patients had hematopoiesis from only a single cord by 6 weeks, which persisted out to day 100. By Day +100, one cord blood unit predominated in all patients.

We have investigated further the characteristics of the predominant or "winning" cord blood unit. Barker and colleagues found no relation between HLA match or cell dose and cord blood predominance. Interestingly, a higher CD3+ dose was associated with cord blood predominance. In our series, the predominant cord blood was the first cord blood infused in 76% of patients. There was a trend to a higher CD34+ cells dose and nucleated cell count in the predominant cord. These interesting observations will need to be tested in a larger cohort of patients.

The use of double cord blood transplantation has decreased the transplant related mortality in adults and does not appear to increase graft vs host disease.
However, infection and immune reconstitution remain significant problems, even with the infusion of two cord blood units.

**Combination cord blood and bone marrow transplants**

The idea of this approach is to achieve initial engraftment from a mismatched bone marrow transplant, followed by rejection of the bone marrow, and long term engraftment of the cord blood unit. The Spanish group has pioneered this novel idea. In the initial study, eleven adults received single cord blood units and haploidentical CD34+ selected cells from a family member. Neutrophil engraftment occurred at 12 days (range 9-36 days). Four patients experienced Grade II or higher acute GVHD. Five of the 11 patients survive disease free with complete cord blood chimerism at 6 to 43 months post transplant.

Murine studies indicate increased engraftment with cotransplantation of mesenchymal stromal cells from an additional donor. Using a NOD-scid mouse model, these investigators have shown improved engraftment when two cord blood units were transplanted with bone marrow mesenchymal cells from a third donor. These studies suggest that some combination of one or two cord blood units plus bone marrow may be beneficial. Ramirez and colleagues have reported a short period of neutropenia in mice treated with human peripheral blood stem cells and cord blood cells.

There are now alternatives to single cord blood transplantation for adult patients, and outcomes for adult patients are improving. The double cord blood results appear promising in terms of neutrophil recovery, and a low risk of GVHD, but are more expensive because of the additional cord blood unit required. This approach should be tested in larger studies with diverse patient populations. In the United States the Cancer and Leukemia Groups B (CALGB) is embarking on such a study using the reduced intensity conditioning regimen on fludarabine, melphalan, and thymoglobulin piloted at Massachusetts General Hospital in Boston. Finally, the Spanish approach of combined bone marrow and cord blood unit is exciting, particularly for patients who are unable to locate two appropriately matched cord blood units. Patients who do not have a matched sibling donor should search aggressively for either unrelated bone marrow and unrelated cord blood in all national and international registries. Patients without time to find an unrelated bone marrow donor, or who do not have a 10/10 or 9/10 unrelated adult volunteer donor should be considered for cord blood transplant. In adults, the double cord blood transplant approach should be strongly considered. The goal should be to procure a safe donor source, either bone marrow or cord blood, in a timely fashion so no patients are denied potentially curative transplant therapy.

**New Applications of Cord Blood Transplantation**
The next 5-10 years should be an exciting time in the cord blood transplantation field. Further investigation is needed to compare cord blood with other donor sources, such as haploidentical transplants, autologous transplants, and mismatched unrelated donor transplants. There might be a future indication for cord blood instead of a matched unrelated donor, for example, in elderly patients with a high risk of graft versus host disease.

An exciting opportunity for cord blood transplantation is in non-malignant disease. A possible application might be in the autoimmune diseases, where there are ongoing trials for autologous transplantation for lupus, systemic sclerosis, and multiple sclerosis. Reports of successful allogeneic transplantation for autoimmune disease have recently been published. A potential advantage of cord blood for these non-malignant diseases is the decreased incidence of graft vs host disease.

Perhaps the most exciting uses of umbilical cord blood might be in cardiac or neurologic disease. Cord blood cells are a more primitive population than adult bone marrow, and have increased capacity for multilineage differentiation. Encouraging preliminary results have been seen in animal models of neurologic and cardiac disease. In a mouse model, cord blood cells injected into the tail vein migrated to infarcted myocardial tissue. Infarct size was smaller in the mice treated with cord blood cells.

Cord blood cells can be expanded in culture and induced to differentiate into cells with neural markers. Recently, cord blood cells have been shown to improve functional recovery in rats that have been subjected to strokes, by middle cerebral artery occlusion. Infarct volume was reduced and behavioral performance increased when a higher dose of cord blood cells was infused.

Conclusion

Umbilical cord blood, traditionally a discarded waste product after delivery, has now become an alternative stem cell source for patients without matched related donors. Transplant outcomes in children are similar to the results seen with unrelated donor transplants. Traditionally, adult transplant has been limited by cell dose and an increased risk of infection. However, new techniques, such as double cord blood transplantation, may help to improve engraftment and immune reconstitution. Particularly intriguing is the interest in cord blood for the treatment of nonmalignant disease. We anticipate continued growth in this exciting field over the next five to ten years.

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blood and mobilized peripheral blood co-transplanted into NOD/SCID mice. Bone Marrow Transplant 2005; 35:271-5.


Table 1  Adult Cord Blood Transplant—Single Unit Ablative Regimen

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<td>Cy/TBI or Bu/CY/ATG</td>
<td>22</td>
<td>26%</td>
</tr>
<tr>
<td>Investigator</td>
<td>N</td>
<td>Diseases</td>
<td>Conditioning Regimen</td>
<td>Median Follow-up</td>
<td>Disease Free Survival (%)</td>
</tr>
<tr>
<td>--------------</td>
<td>-----</td>
<td>-----------------------</td>
<td>---------------------------------------</td>
<td>------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td>Miyakoshi</td>
<td>30</td>
<td>AML, ALL, CML, MDS, lymphoma</td>
<td>Fludarabine, Melphalan, low dose TBI</td>
<td>8</td>
<td>33%</td>
</tr>
<tr>
<td>Chao</td>
<td>13</td>
<td>AML, ALL, MDS, Lymphoma</td>
<td>Fludarabine, Cyclophosphamide, ATG</td>
<td>20</td>
<td>24%</td>
</tr>
<tr>
<td>Yuji</td>
<td>20</td>
<td>Lymphoma</td>
<td>Fludarabine, Melphalan, low dose TBI</td>
<td>12</td>
<td>50%</td>
</tr>
</tbody>
</table>

Abbreviations: AML: Acute myelogeneous Leukemia, ALL: Acute Lymphoblastic Leukemia, CML: Chronic myelogeneous Leukemia; MDS: Myelodysplasia. TBI: total body radiation; Cy: cyclophosphamide; ARA-C: cytosine arabinoside; ATG: antithymocyte globulin.

Table 2: Adult Cord Blood Transplantation: Single Unit: Reduced Intensity Regimen.
Table 3: Adult Cord Blood Transplantation: Double Unit: Ablative Regimen

<table>
<thead>
<tr>
<th>Investigator</th>
<th>N</th>
<th>Diseases</th>
<th>Conditioning</th>
<th>Median Followup (Months)</th>
<th>Disease Free Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mao</td>
<td>6</td>
<td>Aplastic Anemia</td>
<td>Cytoxan/ATG</td>
<td>20</td>
<td>67</td>
</tr>
<tr>
<td>Barker</td>
<td>23</td>
<td>AML, ALL, CML, lymphoma</td>
<td>Cy/TBI/Fludarabine</td>
<td>10</td>
<td>31</td>
</tr>
</tbody>
</table>

Abbreviations: AML: Acute myelogeneous Leukemia, ALL: Acute Lymphoblastic Leukemia, CML: Chronic myelogeneous Leukemia; TBI: total body radiation; ATG: antithymocyte globulin

Table 4: Adult Cord Blood Transplantation: Double Unit: Reduced Intensity Regimen

<table>
<thead>
<tr>
<th>Investigator</th>
<th>N</th>
<th>Diseases</th>
<th>Conditioning</th>
<th>Median Follow-up (months)</th>
<th>Disease Free Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barker</td>
<td>24</td>
<td>AML, ALL, CML, MDS, lymphoma</td>
<td>Busulfan/Fludarabine/low dose TBI or Cyclophosphamide/Fludarabine/low dose TBI</td>
<td>18</td>
<td>55%</td>
</tr>
<tr>
<td>Ballen</td>
<td>21</td>
<td>AML, ALL, MDS, lymphoma</td>
<td>Fludarabine, Melphalan, Thymoglobulin</td>
<td>18</td>
<td>55%</td>
</tr>
</tbody>
</table>