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

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Summary

Cord blood, because of its rich mix of fetal and adult hemoglobin, platelet and WBC counts, and a plasma filled with cytokine and growth factors, as well as its hypoantigenic nature and altered metabolic profile, has all the potential of a real and safe alternative to adult blood during emergencies or any etiology of blood loss.

Study design: In the present study transfusion-related CD34 levels of the peripheral blood from six randomly selected patients suffering from advanced clinical Stage IV malignancy were analyzed between 16 August 1999 and 16 May 2001. This study attempts to ascertain 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 in sex- and HLA-randomized patients.

Results and Analysis: Among the six cases, Case 2 (breast sarcoma) received the lowest amount of card blood (6 units), while Case 6 (breast cancer) received the largest amount (32 units). The youngest patient, suffering from non-Hodgkin's lymphoma (Case 3), was a 16-year-old boy who received eight units of cord blood to combat anemia. Other patients received amounts varying from 7-15 units: Case 4 received 15 units (metachronous lymph node metastatsis), Case 1 received 14 units (breast cancer), and Case 5 received seven units (lung cancer). There was no transfusion-related clinical immunological or nonimmunological reaction. Studies of CD34 levels showed an initial rise followed by a fall in two cases, two cases registered very little effect on the CD34 level, i.e., no change from the baseline, and one case demonstrated a very slow rise from the baseline. However, one case showed a frequent steep rise up to 99% and a sustained high CD34 level. This patient is alive with clinical remission of the disease.

Conclusion: It appears from this preliminary study that freshly collected cord blood transfusion may cause a transient transplant impact of transfused cord blood CD34 stem cells on the host without provoking clinical graft vs host disease due to a of background immune suppression in advanced malignancy. The growth factor cytokine system of freshly collected cord blood may have a potentiating role on the immune system of the host.

Key words: Safe; Placental cord whole blood transfusion; Anemia in advanced cancer; Cord blood; Transient transplant effect; CD34 hematopoietic stem cells.

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, dietary enrichment and supplementation, and finally, red blood cell transfusion. Severe anemia can cause subsequent tumor cell hypoxia, which can reduce the tumorocidal effect of radiation in general [3-7]. 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 function. Thus, cancer patients should ideally receive specially processed blood products, such as leukoreduced, irradiated, cytomegalovirus seronegative blood products. Leukoreduction 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 to 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 the search for a solution to the problem of anemia in patients with advanced cancer, my team has 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 (e.g., the cow).

The work was supported by a research grant by the Dept. of Science and Technology, Govt. of West Bengal, Calcutta, India.

Revised manuscript accepted for publication February 2, 2006

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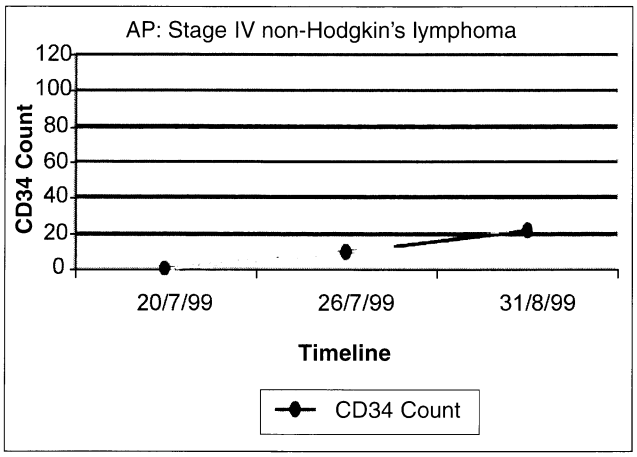

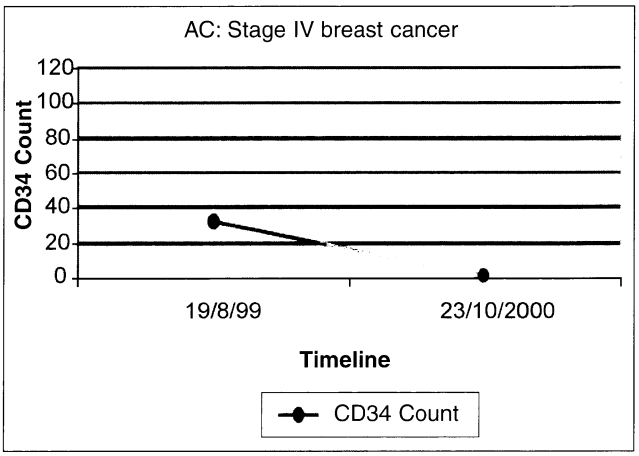

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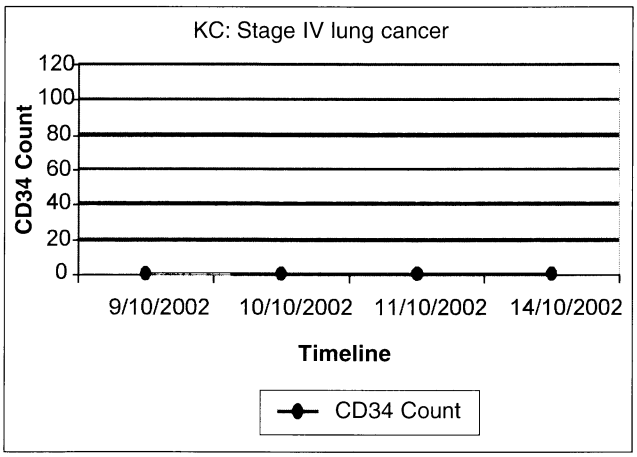


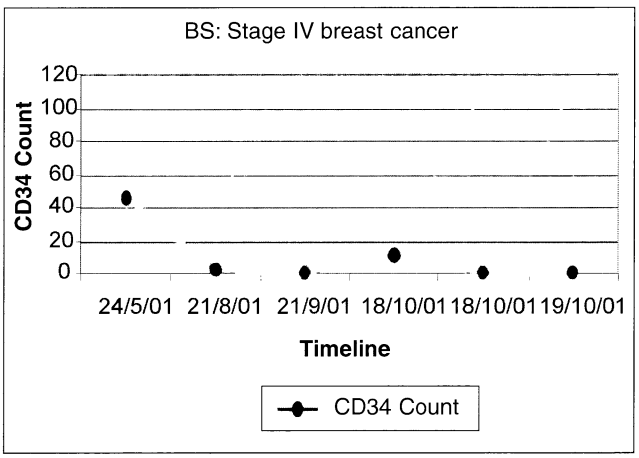


Fig. 4

Figure 1. — Serial CD 34 flow analysis cytometry report of a patient (Case 1) suffering from clinical Stage IV non-Hodgkin's lymphoma.
Figure 2. — Serial CD 34 flow analysis cytometry report of a patient (Case 2) suffering from clinical Stage IV breast carcinoma.
Figure 3. — Serial CD 34 flow analysis cytometry report of a patient (Case 3) suffering from clinical Stage IV lung carcinoma.
Figure 4. — Serial CD 34 flow analysis cytometry report of a patient (Case 4) suffering from clinical Stage IV breast carcinoma.

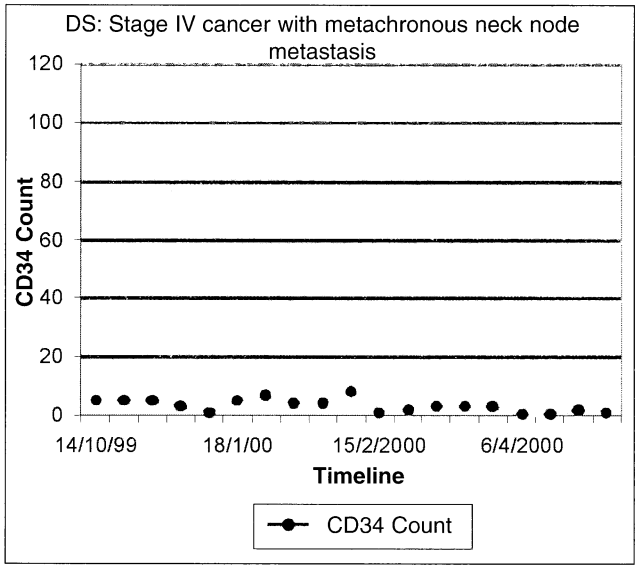
Nature appears to have provided precious wisdom to some of its creatures, but humans seem to be unaware of the positive properties of the womb. There is up to 150 ml of blood in the placenta which has a higher hemoglobin content than adult blood. The high fetal hemoglobin content brings about a normal stress response in pregnancy anemia, thyrotoxicosis, etc., and can also carry more oxygen. If collected aseptically from healthy babies after lower uterine cesarian section (LUCS), this blood can be used as an emergency source of fresh blood for transfusion purposes. It 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 previously reported our experience with the transfusion of 413 units of freshly collected placental umbilical cord blood, in which it was noted that not a single case of immunological or non immunological reaction was encountered [11].

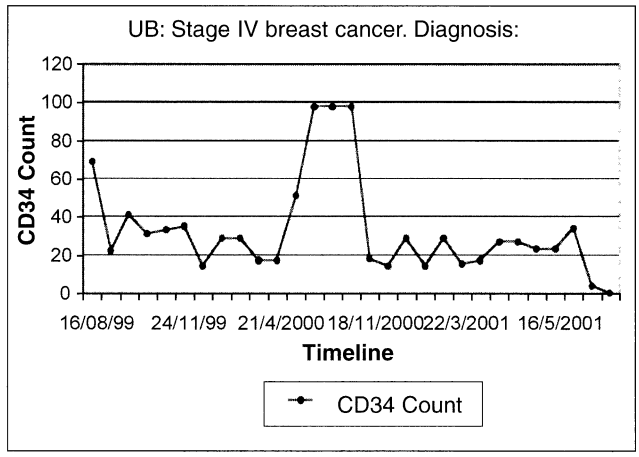
In the present study, we have examined the fate of CD34 in placental blood which was transfused along with all other blood components to advanced cancer patients with anemia (8 g/dl percent hemoglobin or less).

Material and Methods

Human placental umbilical cord blood was collected from consenting mothers aseptically after LUCS 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 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 earlier [12]. Flow analysis cytometry was done routinely to estimate CD34 levels of the peripheral blood three days after the transfusion of cord blood in sex- and HLA-randomized patients. No patient received any growth factor or specific immunosuppressive drug during the cord blood transfusion.

Fig. 5





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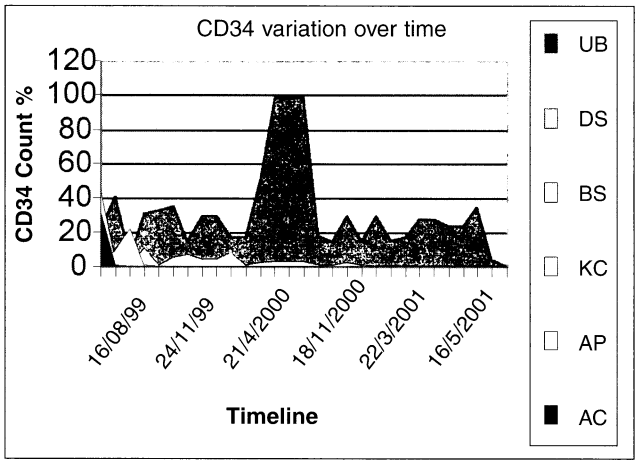

Figure 5. — Serial CD 34 flow analysis cytometry report of patient (Case 5) suffering from clinical Stage 4 neck gland carcinoma.

Figure 6. — Serial CD 34 flow analysis cytometry report of patient (Case 6) suffering from clinical Stage 4 breast carcinoma.

Figure 7. — Comparison of Serial CD 34 flow analysis cytometry report of different patients with advanced malignancy.

Result and Analysis

Six patients with advanced malignancy (Stage IV disease) were enrolled in the study of hematopoietic stem cells (CD34) after placental umbilical cord whole blood transfusion (as assessed from peripheral blood CD34 levels 72 hours after cord blood transfusion). All transfusion-related CD34 levels of the peripheral blood were analyzed between 16 August 1999 and 16 May 2001.

The study group included three males and three females with clinical Stage IV malignancy (Table 1). Each patient also had anemia (< 8 g/dl percent hemoglobin) and received freshly collected placental umbilical cord blood. The patients also received the standard treatment, i.e., surgery, radiation and chemotherapy according to stage and grade of illness in addition to the transfusion protocol.

Case 4, a patient suffering from breast sarcoma, received six units of cord blood, which was the lowest amount transfused in this group (Figure 4). Case 6, who had breast cancer, received the highest amount - 32 units (Figure 6). The youngest patient (Case 1), suffering from non-Hodgkin's lymphoma, was a 16-year-old boy who received eight units of cord blood (Figure 1). Case 5 (metachronous lymph node metastatsis) received 15 units (Figure 5), Case 2 (breast cancer) received 14 units (Figure 2), and Case 3 (lung cancer) received seven units (Figure 3). There were no transfusion-related clinical, immunological or non-immunological reactions.

Periodic assessment of CD34 levels from the peripheral blood revealed the trends noted below.

Case 1: Non-Hodgkin's lymphoma (Figure 1): peripheral blood CD34 levels showed an apparent rising trend up to 24%. The patient died within three months after the last transfusion due to bronchopneumonia.

Case 2: Breast cancer (Figure 2): peripheral blood CD34 levels showed a declining trend from an initial rise after the first transfusion of cord blood. The patient died within two months after the last transfusion.

Case 3: Lung cancer (Figure 3): peripheral blood CD34 levels never crossed the baseline. The patient died within 15 days after the last transfusion.

Case 4: Breast sarcoma (Figure 4): after an initial hike, CD34 levels came down to the base level with slight marginal variation. The patient died within seven months after the last transfusion.

Table 1. — *List of patients with advanced cancer who were followed-up for serial peripheral blood CD34 levels after cord blood transfusions.*

Case 1 (AP) 16 yrs, M	B+ve	Non-Hodgkin's lymphoma Stage IV	8 units first - 11/05/99 last - 13/07/00
Case 2 (AC) 35 yrs, F	A+ve	Breast cancer Stage IV	14 units first - 23/04/99 last - 09/05/01
Case 3 (KC) 45 yrs, F	B+ve	Lung cancer Stage IV	7 units first - 07/06/02 last - 08/10/02
Case 4 (BS) 54 yrs, M	A+ve	Fibro breast sarcoma & diabetes Stage IV	6 units first - 25/04/99 last - 01/08/99
Case 5 (DS) 51 yrs, M	O+ve	Metastatic lymph nodes in the neck Stage IV	15 units first - 28/11/99 last - 16/05/00
Case 6 (UB) 32 yrs, F	B+ve	Breast cancer Stage IV	33 units first - 12/08/99 last - 10/10/02

Case 5: Metachronous bilateral neck node metastasis: there was marginal variation from the baseline (Figure 5). The patient died within 21 days from the last transfusion of cord blood.

Case 6: Breast cancer: there was a substantial rise after practically every unit of transfusion, reaching up to 99% (Figure 6) of peripheral blood CD34 levels (the normal level of peripheral blood CD34 is less than .09%). This patient is living today without any clinical disease.

A comparison of CD34 levels in the six patients is shown is Figure 7. The only patient who is living today and without clinical disease is UB (Case 6). She received the highest number of cord blood transfusions and had a steep rise in CD34 levels after transfusion. In four other cases there was very little variation or a downward trend in CD34 levels after an initial rise. In Case 1 (non-Hodgkin's lymphoma) there was a slow increase, but the level never went over 24% whereas in Case 6, it actually reached 99%.

There are many important factors which decide the fate of the malignancy and the host, i.e., 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 patient 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 bone marrow, or the bone marrow rejuvination by the CD34 rich cord blood transfusion, causes a transplantation effect due to background immune suppression in 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 and is very uncommon in transfusions for elective surgery, sickle cell anemia, thalassemia, and HIV [14, 15]. Long-term white blood cell (WBC) microchimerism of at least two years' duration has been reported in trauma patients receiving fresh non-leukoreduced (non-LR) blood [16]. A better understanding of factors determining clearance versus chimerism of transfused leukocytes is critical in the prevention of alloimmunization and transfusion-induced graft-versus-host disease, and potentially, in 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 in the placenta determines maternal cytokine patterns [18]. In case of tumors, the expression of HLA-G proteins on the surface of primitive melanoma and metastatic cells confers protection from natural killer (NK) cells and cytopathic 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 the transient transplantation impact on the host system. Trophoblast 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 maternofetal interactions [23].

If we study the functional differences between adult peripheral blood stem cell transplantations with umbilical cord blood cell (UCBC) transplantations, the most important factor, apart from intrinsic differences, is the fact that hematopoietic stem cells (HSC) in UCBC transplantations have had a different set of microenvironmental exposures compared to those of adult marrow or the peripheral blood stem cells (PBSC) of adult blood. An example of differences between sources are some of the observed changes in HSC cycle status, gene expression and the adhesive and invasive properties induced by mobilization procedures used to generate PBSC, e.g., granulocyte colony stimulating factor (G-CSF).

Exposure of the hypoantigenic cord blood cells in the placental environment (or exposure to the hypoantigenic cord blood cells nurtured in the placental environment) along with 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 of 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 levels (much higher than the normal level, i.e., up to .09%). 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 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 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 was a transient rise in the CD34 cells of the peripheral blood up to 99% in the bone marrow in one case 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. On the other hand, non-fluctuation of the CD34 level after cord blood transfusion resulted in early death (Case 3 and Case 5). The pathophysiology and clinical significance of this phenomenon is currently under scientific scrutiny.

Acknowledgements

Many thanks to S.K. Misra, M.D., MIC Health and Family Welfare, Govt. of West Bengal, for constant encouragement and Ranbaxy Laboratory for materials.

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