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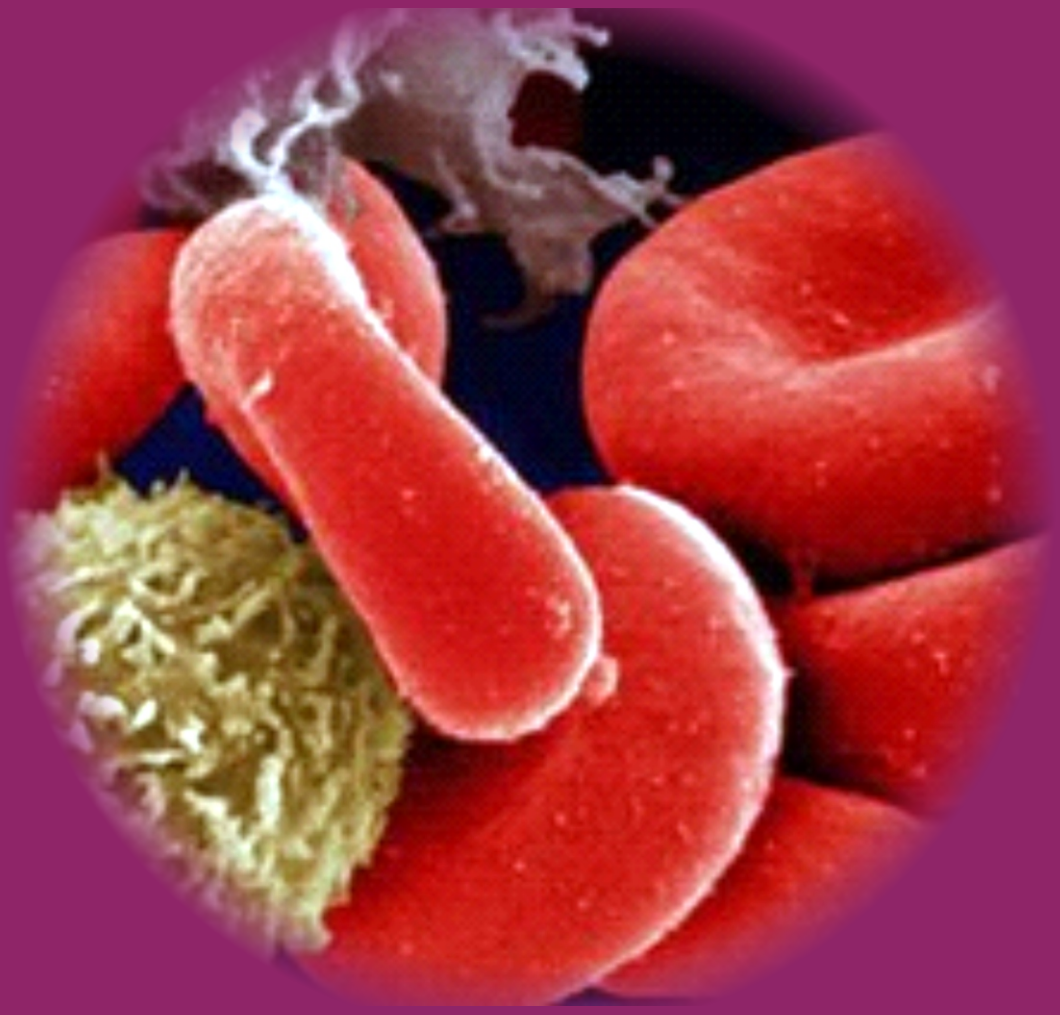

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ORIGINAL RESEARCH ARTICLE

Nigerians with Sickle Cell Trait (Hb AS) have more Haematopoietic Stem Cells in Circulation than those with Hb AA

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ABSTRACT

Background:

The outcome of haematopoietic stem cell (HSC) transplantation depends on the yield of CD34+ cells among other factors, but correlates of CD34+ cells have not been studied among Nigerians.

Aim and Objectives:

This study determined the CD34+ cell count, haematological profile of cord blood and peripheral blood of apparently healthy non-mobilized Nigerian adults; and assessed the relationship between these parameters.

Materials and Methods:

Fifty apparently healthy non-mobilized adults and 50 apparently healthy women in the third trimester of pregnancy were studied after informed consent. Full blood count (FBC) was obtained from peripheral blood of adults and cord blood (SFRI-H18 Medical Diagnostic) together with CD34+ cell count (Cyflow; Partec SL-3).

Results:

The mean CD34+ cell count of cord blood (8.71 ± 8.87/μL) was significantly higher than that of adult blood (1.90 ± 1.43/μL; p = 0.0001). Adults with sickle cell trait (SCT; Hb AS; 18%) had significantly higher CD34+ cell counts (2.92 ± 1.75/μL) than Hb AA individuals (1.68 ± 1.27/μL; p = 0.016). Cord blood of newborns from mothers with SCT (32%) had a significantly higher CD34+ cell counts (12.32 ± 11.27/μL) than those with Hb AA (7.01 ± 7.05/μL; p = 0.047). Cord blood CD34+ cell count weakly correlated with Hb concentration (r = 0.300; p = 0.034) and Hct (r = 0.335; p = 0.017). Adult blood CD34+ cell count weakly correlated, but inversely with platelet count (r = -0.326; p = 0.021).

Conclusion:

Non-mobilized adults and newborns of mothers with SCT are more likely to have a higher CD34+ cell yield than those with Hb AA.

Keywords: Haemopoietic stem cells (CD34+ cells), sickle cell trait (SCT), peripheral blood, cord blood, CD34+ cell yield.

INTRODUCTION

Stem cells were first discovered by Ernest McCulloch and James Till in 1961 who while studying the haematopoietic system of mice observed that immature single cells obtained from mice bone marrow could proliferate and give rise to different types of blood cells [1]. Stem cells have the capacity to develop into many different types of specialized cells in the body, depending on their environment. Haematopoietic stem cells (HSC) are CD34+ cells that are multipotent, giving rise to the different blood cells, usually in the bone marrow under the influence of colony stimulating factors. Haematopoietic stem cells may be used to cure or palliate some malignancies and haematological diseases through allogeneic or autologous transplantation [2].

There are three major sources of HSC i.e. bone marrow (BM), peripheral blood (PB) and cord blood (CB). Peripheral blood stem cells (PBSCs) are small quantities of HSC that migrate from the BM into the peripheral circulation. Administration of G-CSF to donors

for 2-5 days before the harvest, mobilizes more HSC from the BM into the PB, while cord blood may provide a good harvest of HSCs for transplantation, but there may be a need to use more than one donor in this case. [3, 4] Since the harvest of HSC from the bone marrow is an invasive procedure that is conducted usually under general anaesthesia, PB has become a preferred source, notwithstanding the disadvantages like poor venous access, lower HSC yield, side effects of granulocyte-colony stimulating factor (G-CSF) and higher risk of chronic graft versus host disease (GvHD). The quantity and viability of HSCs are crucial to the outcome of haematopoietic cell transplantation (HCT), but the quantification involves the use of flow cytometry, which is expensive and may not be readily available in this environment.

This study was therefore aimed at determining haematological correlates of CD34+ cells among adult Nigerians and in cord blood that may serve as a readily available and affordable surrogate.

MATERIALS AND METHODS

The study was conducted at the Departments of Haematology and Immunology; and Obstetrics and Gynaecology, Obafemi Awolowo University and Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria from November 2013 to July 2014. A study population of 50 apparently healthy adult volunteers (36 males and 14 females) from the Obafemi Awolowo University (OAU) community and 50 healthy women in the third trimester of pregnancy from the ante-natal clinic (ANC) of OAUTHC, Ile-Ife, were purposively recruited for the study after informed consent was obtained. Ethical approval was obtained from the Ethical Review Committee of the OAUTHC, Ile-Ife (Protocol Number ERC/2013/11/01).

Five milliliters (5ml) of venous blood (peripheral) and 5ml of cord blood were collected in EDTA from 50 apparently healthy volunteers and 50 apparently healthy pregnant women at delivery respectively. Whole blood was filtered through 30µm nylon mesh; buffy coat was extracted after centrifugation at 3000 rpm for 25 minutes. The buffy coat was processed by washing with buffer and the CD34+ cells were extracted by magnetically labelling cells with CD34 microbeads, stained with fluorochrome conjugated CD34 antibody and extracted using the MiniMAC Separator following standard operating procedures of the manufacturer (Miltenyl Biotech). Flow cytometry (Partec SL-3) was used to count the CD34+ labelled cells at the Institute of Child Health, University College Hospital (UCH), Ibadan, Nigeria. An automated haematology analyzer (SFRI-H18 Medical Diagnostic; Serial Number: 02010843; France) was used to obtain the full blood count (FBC) and white cell differentials of adult and cord blood.

The haemoglobin (Hb) type of mothers of newborns were obtained from the case notes while that of apparently healthy adults in the OAU community were obtained from subjects who had evidence of their haemoglobin type i.e. driver's licence and previous laboratory results. Haemoglobin types are determined in this institution using haemoglobin electrophoresis method (cellulose acetate paper at alkaline pH). Apparently healthy subjects with no history of any illness and apparently healthy pregnant women in the third trimester where recruited. Individuals with haemoglobinopathies e.g. sickle cell anemia (SCA) and known medical ailment like hypertension, AIDS, diabetes mellitus were excluded from the study. Data were analysed using descriptive and inferential statistics (SPSS version 20); P-value $\leqslant$ 0.05 was used to define statistical significance.

RESULTS

The adults were 32.2 ± 9.7 years of age with, M: F ratio of 2.6:1 while the newborns had M: F ratio of 1: 1.1 ($P > 0.05$). The mean CD34+ cell count of cord blood (8.71 ± 8.87/µL) was significantly higher than that of adult blood (1.90 ± 1.43/µL; $p = 0.0001$; Figure 1).

Adults with sickle cell trait (Hb AS, 18%) had significantly higher CD34+ cell counts (2.92 ± 1.75/µL) than individuals with Hb AA (1.68 ± 1.27/µL; $p = 0.016$), likewise cord blood of newborns from mothers with Hb AS (32%) had

a significantly higher mean CD34+ cell count (12.32 ± 11.27/µL; Figure 2) than those with Hb AA (7.01 ± 7.05/µL; p = 0.047). Figures 3A and 3B and 4A and 4B show the cyflow diagrams for an Hb AA versus Hb AS adult and from cord blood of a newborn from an Hb AA versus Hb AS mother, respectively. Cord blood CD34+ cell count weakly correlated with cord Hb concentration (r = 0.300; p = 0.034; Figure 5) and cord blood haematocrit (Hct; r = 0.335; p = 0.017; Figure 6). Adult blood CD34+ cell count also weakly correlated, but inversely with platelet count (r = -0.326; p = 0.021; Figure 7).

DISCUSSION

Various studies have reported the effect of donor characteristics like gender, age, gestational age and weight of donors on the yield of CD34+ cell count, but none from this environment. [5, 6] This is the first report of the quantification of CD34+ HSCs in cord blood and peripheral blood in relation to Hb types (AA and AS) among Nigerians. The mean CD34+ cell count of cord blood was significantly higher than that of adult blood (Figure 1). This study confirms a report that relatively higher numbers of HSCs are found in cord blood when compared to peripheral blood of adults [7].

The prevalence of the Hb types in this study (Figure 2) is similar to those reported by others that showed the prevalence of Hb AA to vary between 55-75%, while the Hb AS varies between 20-30% in Nigeria [8, 9]. Adults with Hb AS yielded a significantly higher CD34+ cell count than Hb AA individuals (*p = 0.016*) and cord blood of newborns from mothers with SCT were also significantly higher than those of mothers with Hb AA (*p = 0.047*; Figure 2). The Hb type of mothers in this study may therefore predict the yield of CD34+ cells that may be harvested from cord blood. To the best of the knowledge of the authors, this is the first report of this relationship in literature and therefore, further studies would be needed to confirm this observation. Among adults, the significant difference obtained in this study between Hb AA and Hb AS individuals (Figure 2) did not confirm the observations of Panch *et al*, (2016) who studied racial differences in the yield of CD34+ cells among Caucasians and Afro-Americans. [10] The difference might have been masked by the use of G-CSF, which was not used in this study to mobilize these cells from the BM.

A weak positive correlation was obtained between CD34+ cell counts and Hb concentration (Figure 5) and PCV (Figure 6) in cord blood, but the strength of these correlations were not sufficient to make Hct or Hb concentration surrogate markers of CD34+ cell counts in cord blood. In the peripheral blood of apparently healthy adults, however, the relationship between CD34+ cell counts and Hct and Hb concentration obtained in this study confirms the report that there was no correlation between CD34+ cells and Hct [11].

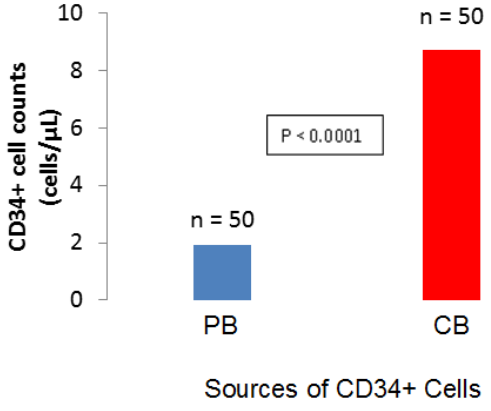


Figure 1: The mean CD34+ cell count of peripheral blood of adults compared with cord blood

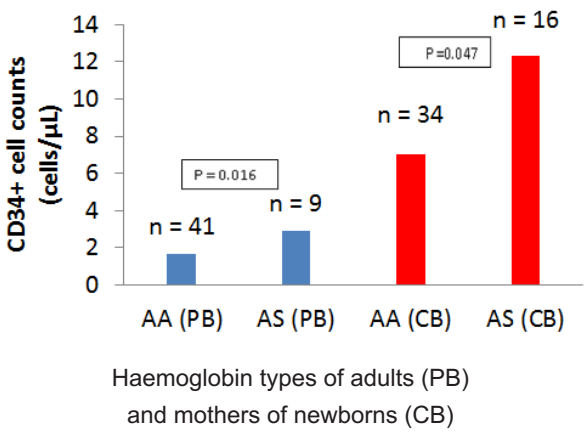
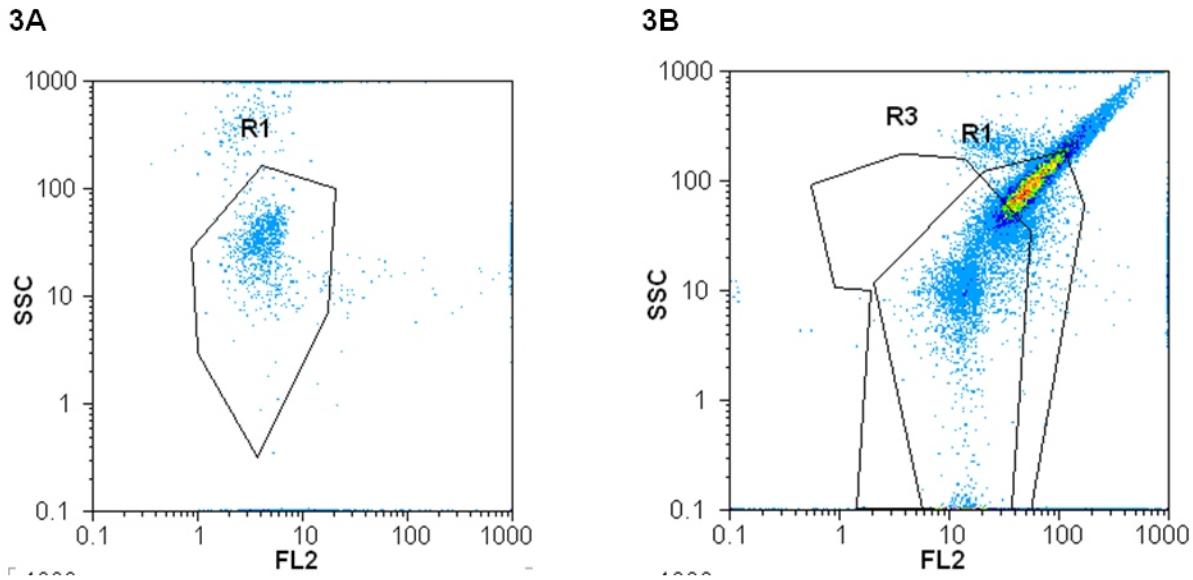


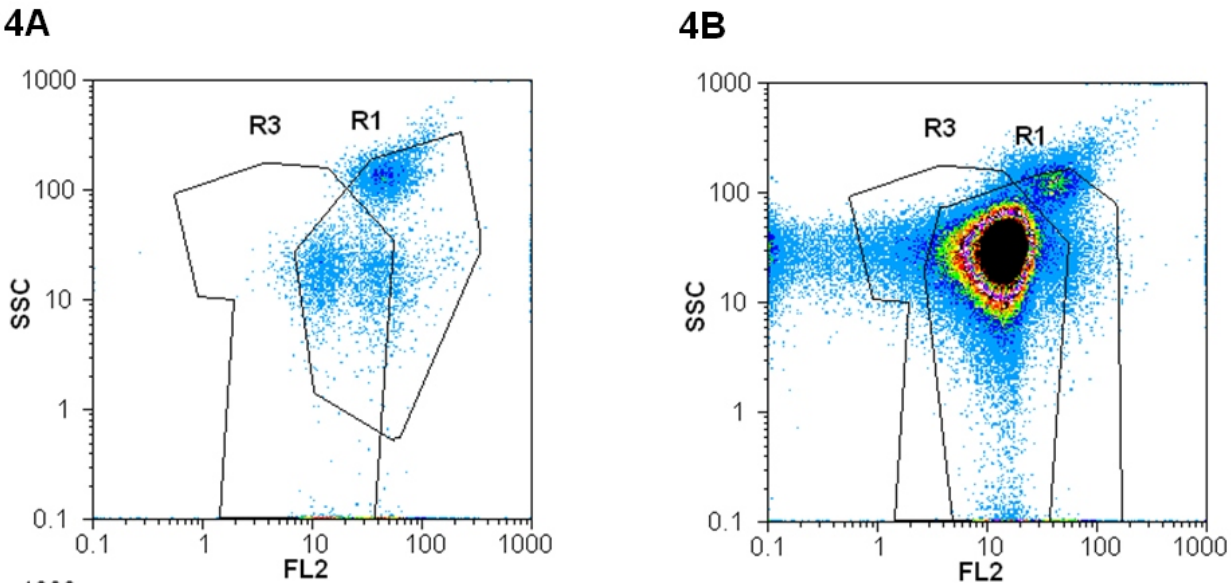
Figure 2: The CD34+ cell count in peripheral blood (PB) and cord blood (CB) by haemoglobin type



Key: SSC - Side Scatter Light; FL2 – quantification of fluorescent CD34+ cells; R1 and R3 – gating polygons

Figure 3A: Flow Cytometric Enumeration of CD34+ Cells in the Peripheral Blood of an Hb AA Adult (Subject P1 CD 34+ cells = 1.072/μL) Side Scatter Light (SSC) vs. CD34/FL2. True CD34 events form a discrete cluster, and a region is set around this population (R1)

Figure 3B: Flow Cytometric Enumeration of CD34+ Cells in the Peripheral Blood of an Hb AS Adult (Subject P50 CD34+ cells = 4.99/μL) SSC vs. CD34/FL2. True CD34 events form a discrete cluster, and a region is set around this population (R1/R3)



Key: SSC - Side Scatter Light; FL2 – quantification of fluorescent CD34+ cells; R1 and R3 – gating polygons

Figure 4A: Flow Cytometric Enumeration of CD34+ Cells in the Cord Blood of a Newborn from Hb AA Mother (Subject C02 CD34+ cells = 2.45/μL) SSC vs. CD34/FL2. True CD34 events form a discrete cluster, and a region is set around this population (R1/R3)

Figure 4B: Flow Cytometric Enumeration of CD34+ Cells in the Cord Blood of a Newborn from Hb AS Mother (Subject C30 CD34+ cells = 44.19/μL) SSC vs. CD34/FL2. True CD34 events form a discrete cluster, and a region is set around this population (R1/R3)

It is uncommon that both Hct and Hb concentration correlated with CD34+ cell count in cord blood only, because there is normally a relationship between these two red cell indices. This may be due to varying clamp times that have been reported to influence the yield of HSC in cord blood [12].

A weak inverse relationship was obtained between CD34+ cells and platelet counts in peripheral blood of healthy adults in this study (r = -0.326; P = 0.021; Figure 7). Although this relationship agrees with the findings of Knudsen *et al* (1999), the platelet count cannot be used as a surrogate marker for CD34+ cell counts in adults because the relationship was not strong enough. [13] Again, the relationship between CD34+ cell count and platelets in the peripheral blood of adults was not mirrored by the counts in cord blood. The reasons for these relationships are not clear and none reached surrogate marker status. Since no surrogate marker for CD34+ cell count was found in this study, the quantification of CD34+ cells must therefore be obtained with the use of flow cytometry until other means of quantifying HSC

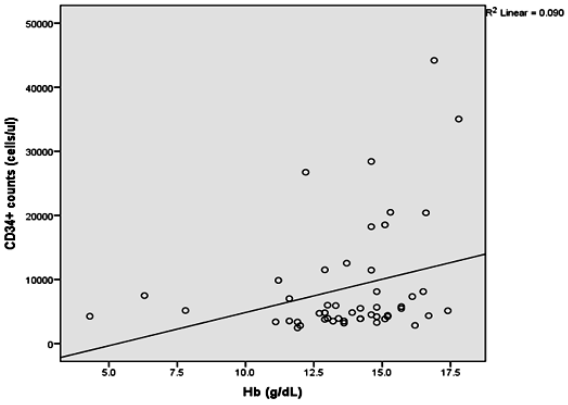


Figure 5: Correlation between CD34+ cell count and haemoglobin (Hb) concentration in cord blood (r = 0.300; p = 0.034).

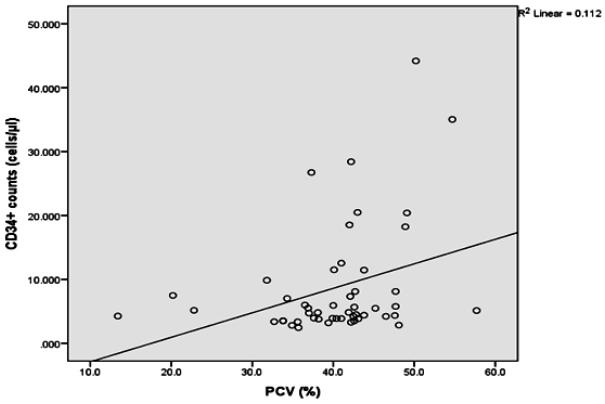


Figure 6: Correlation between CD34+ cell count and packed cell volume (PCV) in cord blood (r = 0.335; p = 0.017).

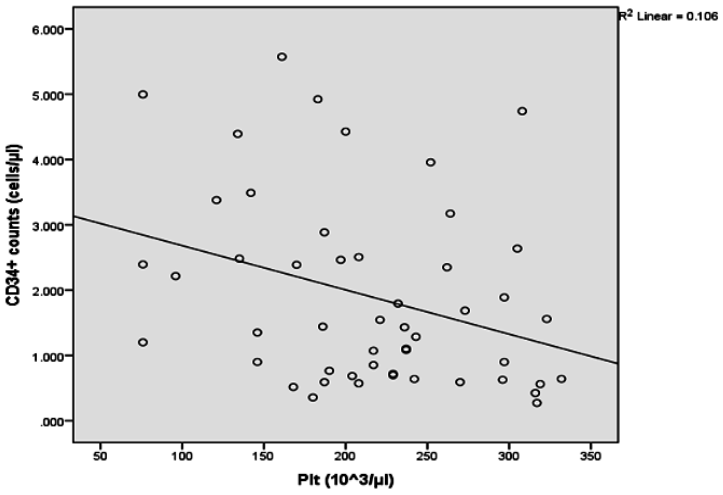


Figure 7: Correlation between CD34+ cell counts and platelet (Plt) counts in the peripheral blood (PB) of adults (r = -0.326; p = 0.021).

are available. This method is expensive, but remains the only method to confirm adequate volumes of harvests from any haematopoietic stem cell source, even in a resource limited setting like Nigeria. Individuals with SCT or cord blood from newborns of mothers with Hb AS may provide a relatively increased yield when compared to their Hb AA counterparts.

In conclusion, this study has confirmed that cord blood is a better source of HSC (CD34+) cells than adult blood per unit volume in non-mobilized apparently healthy Nigerians. Peripheral blood of non-mobilized adults with SCT and cord blood of newborns of mothers with SCT are more likely to have a higher CD34+ cell yield than those with Hb AA. There was a tendency for CD34+ cells to increase as haemoglobin concentration and haematocrit of cord blood increased, but as platelet count of non-mobilized adult blood decreased. The eligibility of individuals with SCT as organ donors for transplantation should be further investigated.

Acknowledgement:

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Conflict of Interest:

The authors have not declared any conflict of interest.

Author's Contributions:

SA contributed to the design of the study, purchase of reagents, conducted the tests and contributed to the writing of the paper, whilst NOA designed, purchased the reagents, analysed the data and contributed to the writing of the paper. AO contributed to the design, purchase of the reagents and writing of the paper.

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