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Cellular changes in stored whole blood and the implication on efficacy of transfusion therapy in Nigeria

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ABSTRACT: Due to inadequacy of fractionation technology, whole blood is still frequently used in Nigeria. This study was conducted to determine the extent to which whole blood maybe a useful source of leucocytes and/or platelets. A unit of whole blood was collected (in CPDA-1 bag) from a Nigerian donor. Serial blood counts were determined from day of donation till expiry (day-35). The values before refrigeration were: haematocrit 40.1%, leucocyte $6.7x10^9$/l and platelet $253x10^9$/l, which fell during 4^oC storage to 35.1%, $0.2x10^9$/l and $35x10^9$/l on day-35. The leucocytes and platelets counts fell below the critical values of 3 $x10^9$/l and 100 $x10^9$/l, which corresponded to the lower limits for leucocyte and platelet counts in Nigerians, after the 2nd day of storage. This study suggests that the potential usefulness of stored whole blood as a source of leucocytes and/or platelets was limited to the first 2 days after collection.

Key Words: Blood transfusion; Whole blood; Blood fractionation; Platelets; Leucocytes; Haematocrit.

Introduction

Whole blood is a complex tissue comprising cellular and non-cellular components that serve diverse functions. The non-cellular components consist of the plasma and its derivatives [1]. The cellular components consist of leucocytes, platelets and red cells. With advancement in blood fractionation technology it is now possible to harvest each of these components as separate concentrates [1]. This ensures precision and accuracy in clinical transfusion therapy so that individual patients can be given only those components they require [1]. Component therapy has a distributive advantage since a single unit of blood can be fractionated to the benefit of more than one patient [1]. Because of its efficacy, blood component therapy has become the established modality in modern transfusion medicine in the developed countries where whole blood is now rarely used. However, in many developing nations including Nigeria, blood fractionation technology is still at its infancy and restricted to a few health care outlets at tertiary levels.

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The national blood transfusion service in Nigeria is still in a fledgling stage and does not have sufficient functional capability to satisfy the national requirements for blood and blood components. Consequently, Nigerian hospital blood banks still bear the overwhelming and daunting responsibility of donor recruitment as well as production and storage of blood and blood components [2, 3]. This is particularly difficult since donor blood is always in short supply and inadequate with respect to clinical requirements in Nigeria [2, 3]. These circumstances have led to sub-optimal production and utilization of blood components. It is therefore not surprising that whole blood stored at 4^oC is still frequently used and has remained the most commonly used blood product in many hospitals in Nigeria [4]. Stored whole blood units were often used within the context of the 'freshest' blood available in the blood bank at the time of request in an attempt to correct quantitative cellular deficits such as neutropenia and/or thrombocytopenia in patients with various disorders such as aplastic anaemia and other forms of marrow failure, haematological and non-haematological malignancies with marrow affectations and consumptive coagulopathies [4]. However, stored whole blood has several limitations as a source of leucocytes and platelets since the concentrations of these cells fall as the length of storage increases.

In this report we studied the trend in the levels of haematocrit, leucocyte and platelet counts in whole blood from the day of donation till the day of expiry, with the aim of determining the precise pattern and magnitude of fall of each parameter. This will eventually high light the usefulness and limitations of stored whole blood as a source of leucocytes and/or platelets in transfusion therapy for patients with leucopenia and/or thrombocytopenia in Nigeria.

Materials and Methods

A healthy male voluntary blood donor was bled on the 21/01/2008 at the blood bank of Aminu Kano Teaching Hospital, Kano, Nigeria. A sample of blood (2 ml) was taken into ethylenediaminetetraacetatic acid container prior to the blood donation (pre-donation sample) and the full blood count was immediately determined. Subsequently, four hundred and fifty milliliters (450 ml) of blood was taken into CPDA-1 bag (Shandong, China) containing 63 ml of Citrate Phosphate Dextrose Adenine anticoagulant with a maximum storage period of 35 days. During the procedure, the blood was regularly mixed to ensure adequate mixing with anticoagulant and to prevent clot formation [5]. Immediately after completion of blood donation and before refrigeration, 1ml of blood was collected from the outlet of the blood bag using syringes with 16G needles and the full blood count was determined; this pre-refrigeration sample was designated day-0 sample. The blood unit was then transferred to 4^oC blood bank fridge. Thereafter, samples were collected for full blood count at 2-day intervals on days-2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34 and day-35. Each sample was taken on exactly the same hour of the sampling day in order to ensure that the samples were taken exactly 48 hours apart, except the last (day-35) sample that was taken 24 hours after the day-34 sample. In this study, the full blood counts of all samples were determined using Celltac Alpha (MEK 6400) blood analyzer.

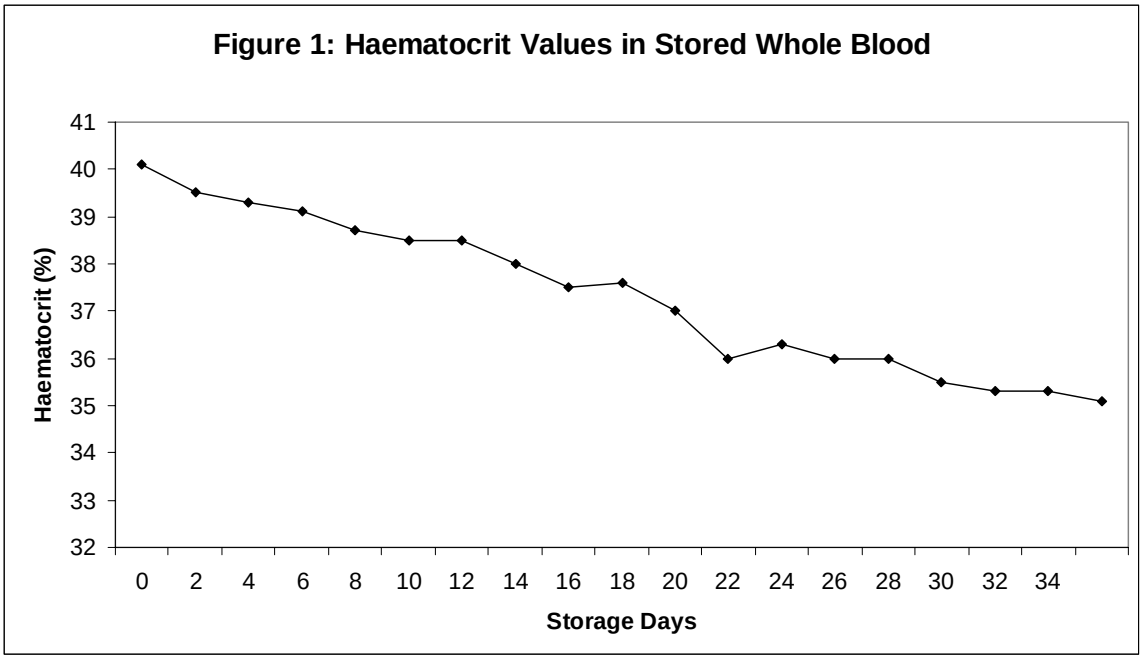
Results

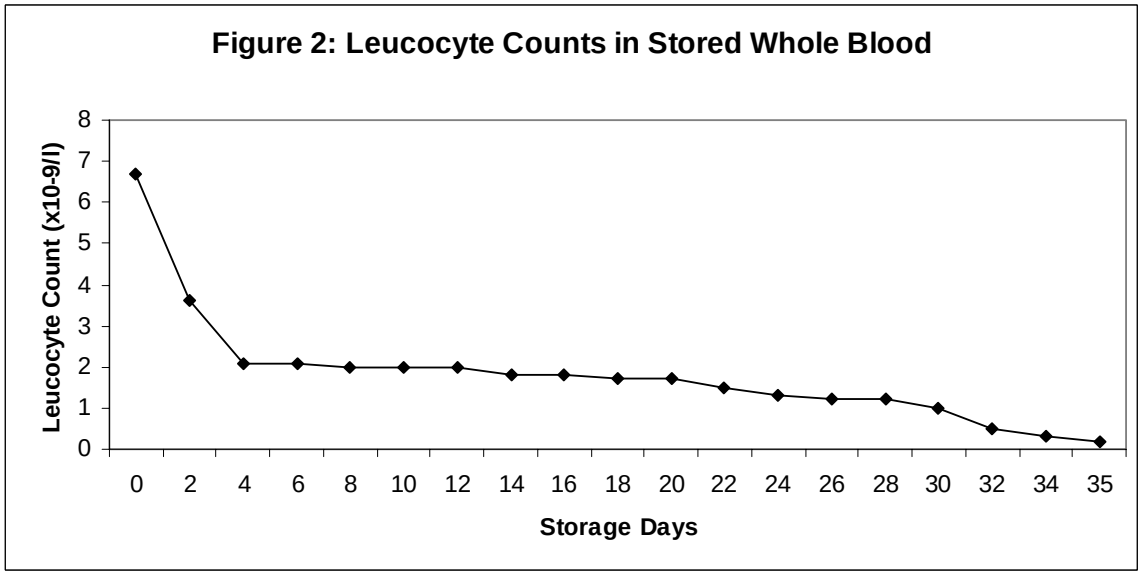
The results were documented as shown in Table 1 and Figures 1-4. The pre-donation haematological parameters of the donor revealed a haematocrit of 44.5%, leucocyte count of 7.3 $x10^9$/l (granulocytes 52%, lymphocytes 41% and monocytes 7%) and platelet count of 285$x10^9$/l. The pre-refrigeration (Day-0) counts showed a haematocrit of 40.1%, leucocyte count of 6.7$x10^9$/l (granulocytes 51.9%, lymphocytes 40.9% and monocytes 7.2%) and platelet count of 253 $x10^9$/l. The haematocrit, leucocytes and platelets fell from 40.1%, 6.7 $x10^9$/l and 253 $x10^9$/l on day-0 to 35.1%, 0.2 $x10^9$/l and 35 $x10^9$/l on day-35 (Table 1 and Figures 1, 2, 3). The percentage falls from day-0 to day-35 were 12.5% for haematocrit, 97% for leucocytes and 86.2% for platelets. The fall in leucocyte count was associated with reversal of granulocytes to lymphocytes ratios as revealed by the relative rise in the proportion of lymphocytes in the serial differential counts from day-2 onwards; the differential monocyte count showed a steady fall that was not as severe as that seen in the granulocytes (Table 1 and Figure 4). The rate of fall for both leucocytes and platelets were

more rapid during the first 4 days as suggested by the steepness of the slopes in Figures 2 & 3, whereas the rate of fall in haematocrit was gradual with no early rapid phase as shown in Figure 1.

Table 1: Haematological Parameters of Pre-donation and Stored Whole Blood Samples

Storage days	Haematocrit (%)	Leucocyte count $x10^9/l$	Granulocytes (%)	Lymphocytes (%)	Monocytes (%)	Platelet count $x10^9/l$
Pre-donation blood count	44.5	7.3	52	41	7	285
Day-0	40.1	6.7	51.9	40.9	7.2	253
Day-2	39.5	3.6	21.1	71.3	7.6	105
Day-4	39.3	2.1	15	78	7	75
Day-6	39.1	2.1	7.8	85.8	6.4	63
Day-8	38.7	2	7.5	85.9	6.6	60
Day-10	38.5	2	7	86.5	6.5	60
Day-12	38.5	2	6.7	86.8	6.5	53
Day-14	38	1.8	5.8	88.7	5.5	51
Day-16	37.5	1.8	4.5	90	5.5	48
Day-18	37.6	1.7	4.3	90.2	5.5	45
Day-20	37	1.7	4	90.5	5.5	41
Day-22	36	1.5	4	90.6	5.4	40
Day-24	36.3	1.3	2.8	91.6	5.6	38
Day-26	36	1.2	1.5	92.9	5.6	37
Day-28	36	1.2	1.5	92.9	5.6	36
Day-30	35.5	1	1	93.4	5.6	36
Day-32	35.3	0.5	0.5	94	5.5	36
Day-34	35.3	0.3	0.5	94	5.5	35
Day-35	35.1	0.2	0.2	94.8	5	35





Discussion

The pre-donation haematological parameters of the donor were consistent with values obtainable in healthy voluntary donors in Nigeria [6]. The values of haematological parameters seen in the day-0 sample taken from the unrefrigerated whole blood unit were slightly lower than the values of the pre-donation blood sample of the donor, a phenomenon that was interpreted be as a result of the dilutional effect of the anticoagulant in the donor bag. The haematocrit fell by only 12.5% during the entire storage period. This gradual but steady fall in haematocrit during storage was an expected finding that was due to depletion of red cell ATP. Previous studies had linked ATP depletion to loss of membrane function, reduced cell

viability and rising levels of potassium and free haemoglobin in the plasma of stored whole blood [7]. The adenine component of the anticoagulant CPDA-1 was strategically added to provide a substrate for the synthesis ATP, hence prolonging the shelf life of stored blood to 35 days, which is longer than with non-adenine containing anticoagulants [8].

Figure 3: Platelet Counts in Stored Whole Blood

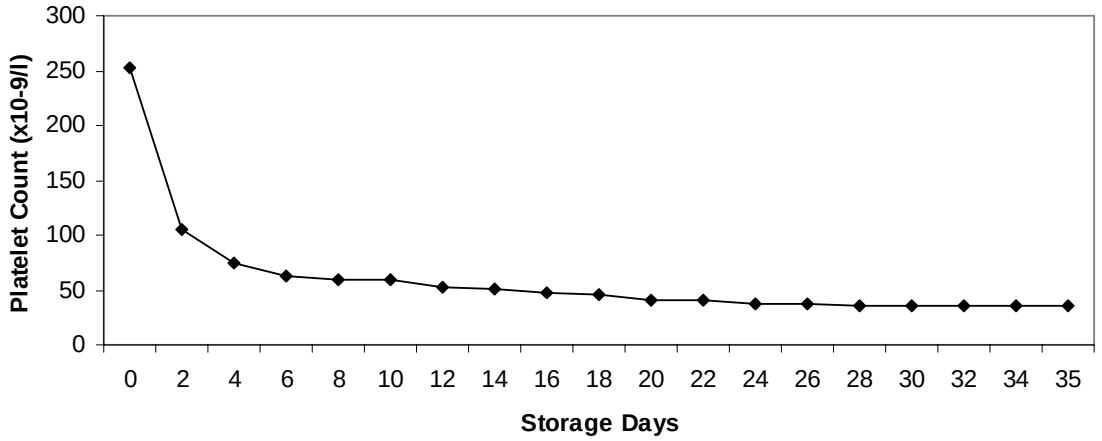
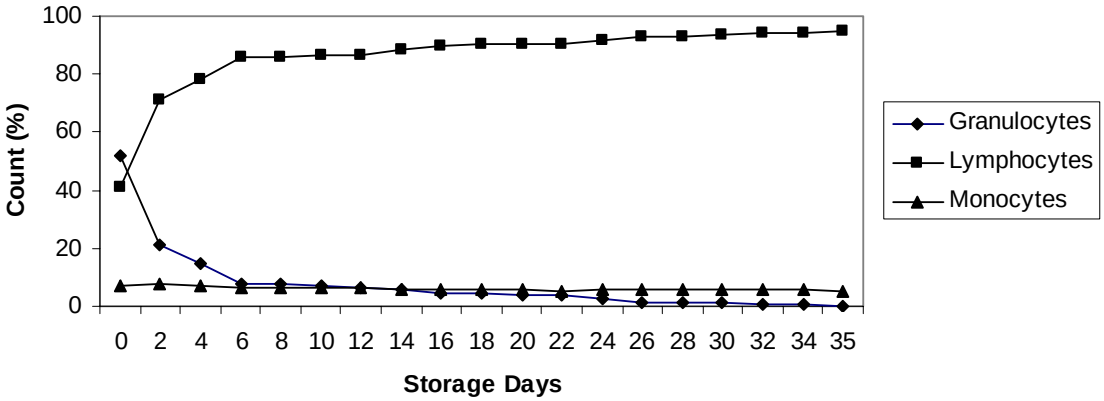


Figure 4: Leucocytes Differential Counts in Stored Whole Blood



In contradistinction to haematocrit, the leucocyte count fell more drastically, by 97%, during the storage period. The mechanism of leucocyte depletion during whole blood storage may include loss of cell viability due to ATP depletion. More over, leucocytes are also consumed in the formation of micro-aggregates, which are conglomerates of leucocytes, platelets, fibrin, cold-insoluble globulin and cellular debris formed during storage [5]. This study revealed a progressive fall in all types of leucocyte however, the pattern of changes observed in the serial differential count would suggest that granulocytes were more labile than the mononuclear cells comprising the lymphocytes and monocytes. The clinical significance of this observation is that stored whole blood would be particularly ineffective as a clinical tool in the management of aplastic anaemia and other leucopenic patients since the most critical entity in these cases is almost always

neutropenia. Further more, this data revealed a specific survival advantage of lymphocytes in stored whole blood, which will imply that stored whole blood carries the risk of graft-versus-host disease if viable donor lymphocytes get engrafted in immuno-deficient recipients and premature neonates [9]. This is particularly important within the context of the current HIV pandemic, which is strongly associated with anaemia and frequent transfusions [10]. The platelets also revealed progressive decline in count, which fell by 86.2% during the period of storage. In similarity to leucocytes, the fall in platelet levels may be related to loss of cell viability due to ATP depletion as well as platelet consumption due to microaggregates formation [5].

The rate of fall of both leucocytes and platelets were faster during the first 4 days, a phenomenon that may probably be suggestive of some degree of cell selection where older and more labile cells died rapidly initially, thus leaving a cohort of younger and more stable cells that died later at much slower rate. However, the rate of fall in haematocrit was gradual with no observable early rapid phase, suggesting greater stability of the red cells relative to leucocytes and platelets under storage conditions. The overall quantitative analysis of this data however, revealed that significant diminution of both leucocyte and platelet counts occurred after day-2 of storage, taking into consideration that the lower limit for leucocyte and platelet counts for the blacks including Nigerians were reported as 3 $x10^9$/l and 100 $x10^9$/l respectively [11, 12]. This would imply that stored whole blood may be useful as a source of both leucocytes and platelets during first 2 days of storage. However, the clinical benefit may be less than anticipated since some studies had suggested that storage at 4^oC would make granulocytes to loose their phagocytic and bactericidal functions after 24 hours and platelets to loose their haemostatic functions within 48 hours [13]. Therefore, the surest way of using whole blood as a source of functional leucocytes and/or platelets is by transfusing it soon after collection without refrigerated storage; the quantitative cell counts of such units were excellent as revealed by the day-0 parameters in this study. A major limiting factor in this setting would be volume overload due to the high plasma to cell ratio in whole blood unit, however, this may be advantageous in patients with acute haemorrhage and hypovolaemia. Another important consideration in using unrefrigerated fresh whole blood is that it has to be transfused within a short time after collection in order to avoid deterioration, a situation that may not permit sufficient time for adequate screening for transfusion transmissible infections. Nonetheless, careful application of well evaluated rapid test kits may be useful in such circumstances [14]. It however must be emphasized that the most efficacious way of correcting neutropenia and/or thrombocytopenia is through blood component therapy [1].

Conclusion

Based on quantitative analysis, the usefulness of stored whole blood as a source of leucocytes and/or platelet was limited to the first 2 days after collection. However, there are concerns regarding the functional capabilities of such leucocytes and platelets. Unrefrigerated fresh whole blood is a better source since it has excellent cell count and the absence of storage guarantees cellular functionality, the main limitation being volume overload. In view of the multiple limitations associated with the use of whole blood, it has become necessary to invigorate the national blood transfusion service in order to develop every aspect of blood fractionation and component therapy in Nigeria.

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