Africa Sanguine

2021, Volume 23, Number 1

https://dx.doi.org/10.4314/asan.v23i1.7

Sickle Cell Trait and the Risk of Clotting in Donated Blood Bags in North West Nigeria: Is this a Call for Pre-Donation Hydration?

Trait drépanocytaire et risque de coagulation dans les sacs de sang donnés dans le nord-ouest du Nigéria: s'agit-il d'un appel à l'hydratation avant le don?

Habib A[1], Ibrahim Z[1], Kani KM[2], Ibrahim UA[3], Ahmed SG[4]

1. Department of Haematology, Rasheed Shekoni Teaching Hospital, Dutse, Nigeria
2. Department of Haematology, Federal Medical Centre, Birnin Kudu, Nigeria
3. Department of Paediatrics, Aminu Kano Teaching Hospital, Kano, Nigeria
4. Department of Haematology, Aminu Kano Teaching Hospital, Kano, Nigeria

Corresponding Author:
Dr Sagir G. Ahmed
Email: drsagirahmed@yahoo.com

Conflict of interest:
The authors have no conflicts of interest to declare

Key words:
Sickle Cell Trait, Blood Donation, Blood Bag Clots, Nigeria

ABSTRACT

Introduction: As a developing nation, Nigeria's transfusion service is characterised by low quality control and a high incidence of clots in blood bags. About one-quarter of donors in Nigeria carry sickle cell trait (SCT), which is haemo-rheologically associated with pathological hyperviscosity and hypercoagulability. Hence, we hypothesised that SCT is a risk factor for clotting in bags, and that the risk would be aggravated by non-O blood groups due to their physiological (natural) hypercoagulability.

Objectives: Determine risk of clotting in bags donated by persons with SCT and its interaction with ABO-blood groups, and proffer possible strategy to mitigate SCT-associated clotting risk in blood bags in Nigeria.

Materials and Methods: Analysis of 100 clotted blood bags. Frequencies of SCT and ABO-groups of clotted and control bags were compared by Student-t and X^2-tests. Risks of clotting for SCT with respect to ABO-groups were expressed as Odds ratios by case-control logistic regression.

Results: Bags with 'SCT and blood group-O' had high clotting risk (OR=1.89, $CI_{95\%}$:1.43-2.34, p=0.031), while bags with 'SCT and non-O groups' had higher clotting risk (OR=2.97, $CI_{95\%}$:2.55-3.45, p=0.015); which suggested risk escalation by non-O groups.

Discussion and Conclusion: SCT is independently associated with high clotting risk, which is synergistically escalated by additional hypercoagulable effects of non-O groups. Clots in blood bags have economic and clinical implications. Hence, blood banks in Nigeria (and other African countries with high prevalence of SCT) should upgrade quality control, routinely screen donors for SCT, and consider feasibility of pre-donation hydration for SCT-positive donors in order to reduce hyperviscosity and clotting risks in blood bags, thereby avoiding blood wastage and shortage.

RÉSUMÉ

Introduction: En tant que pays en développement, le service de transfusion du Nigéria se caractérise par un contrôle de faible qualité et une forte incidence de caillots dans les poches de sang. Environ un quart des donneurs au Nigéria sont porteurs du trait drépanocytaire (SCT), qui est hémo-rhéologique associé à une hyperviscosité et une hypercoagulabilité pathologiques. Par conséquent, nous avons émis l'hypothèse que la SCT est un facteur de risque de coagulation dans des sacs et que le risque serait aggravé par les groupes sanguins non-O en raison de leur hypercoagulabilité physiologique (naturelle).

Objectifs: Déterminer le risque de coagulation dans les sacs donnés par des personnes atteintes de SCT et son interaction avec les groupes sanguins ABO, et proposer une stratégie possible pour atténuer le risque de coagulation associé à la SCT dans les poches de sang au Nigéria.

Matériel et méthodes: Analyse de 100 poches de sang coagulées. Les fréquences des groupes SCT et ABO des poches coagulées et témoins ont été comparées par des tests Student-t et X2. Les risques de coagulation pour la SCT par rapport aux groupes ABO ont été exprimés en odds ratios par régression logistique cas-témoins.

Résultats: Les poches avec `` SCT et groupe sanguin-O " avaient un risque de coagulation élevé (OR = 1,89, IC95%: 1,43-2,34, p = 0,031), tandis que les poches avec `` groupes SCT et non-O " avaient un risque de coagulation plus élevé (OR = 2,97, IC95%: 2,55-3,45, p = 0,015); qui a suggéré une escalade du risque par les groupes non-O.

Discussion et conclusion: La SCT est indépendamment associée à un risque de coagulation élevé, qui est augmenté de manière synergique par des effets hypercoagulables supplémentaires des groupes non-O. Les caillots dans les poches de sang ont des implications économiques et cliniques. Par conséquent, les banques de sang au Nigéria (et dans d'autres pays africains à forte prévalence de SCT) devraient améliorer le contrôle de la qualité, dépister systématiquement les donneurs pour la SCT et envisager la faisabilité de l'hydratation avant le don pour les donneurs SCT positifs afin de réduire l'hyperviscosité et les risques de coagulation chez les poches de sang, évitant ainsi le gaspillage et la pénurie de sang.

INTRODUCTION

Nigeria has the largest population (over 200 million) in Africa, yet the national blood transfusion service is still in an evolving stage and is thus unable to adequately cater for the entire blood need of the nation's hospitals.[1] Consequently, the responsibilities of donor recruitment, selection, and bleeding are essentially relegated to individual hospital blood banks.[1] The eligibility of prospective donors in Nigeria is determined by a pre-donation assessment of health status.[2] The assessment is in the form of oral questioning and/or questionnaire screening with respect to general health, as well as medical and social history, which is followed by simple physical examination and measurements of donor weight and blood pressure.[2] Persons who are between the ages of 18 and 65 years, and have passed the pre-donation medical assessment with haemoglobin (Hb) levels of more than 13.5 g/dL for males or 12.5 g/dL for females (excluding pregnant and lactating women), and have tested negative for HIV, hepatitis B and C viruses, and syphilis are acceptable as donors.[2] Once an eligible donor is identified, the largest vein in the ante-cubital fossa is selected by applying a tourniquet or blood pressure cuff inflated to 40-60 mmHg.[3,4] After meticulous skin cleaning and disinfection, a minimally traumatic single-entry phlebotomy is performed using the 16-gauge needle that is attached to the blood collection bag.[3,4] In order to facilitate and maintain optimal rate of blood flow, the donor is instructed to squeeze a compressible rubber ball or open and close the fist slowly every 10-12 seconds during the period of blood collection.[3,4] After every 30 seconds during the donation, blood is manually mixed with the anticoagulant (citrate-phosphate-dextrose-adenine) in the donation bag until a target volume of 500 mL is collected.[3,4] Normal blood has a specific gravity of about 1.053, hence the target volume of 500 mL would correspond to an average weight of about 500 g (474-579 g),[4] which is usually measured with manual weighing scales in Nigerian blood banks. In Nigeria, the blood collection procedure is entirely manual, which is unlike what is obtainable in developed countries where automated electronic blood collection monitors equipped with auto-flow rate - sensing, auto-mixing, auto-weight-sensing, and auto-clamping facilities are available. The risk of clotting increases with the length of donation time. Hence, blood donation procedure should ideally be completed in less than 10 minutes,[4] and a blood unit drawn over a period longer than 15-20 minutes may not be suitable for making haemostatically active blood components such as platelet concentrates, fresh frozen plasma or cryoprecipitate.[4]

From pathophysiologic perspectives, there are four possible trigger mechanisms (root causes) for coagulation activation and clot formation in blood bags during blood donation. First, an untidy and unduly traumatic venepuncture can cause significant vascular endothelial injury with exposure of sub-endothelial microfibrils and collagen, which can trigger contact activation of platelets and FXII, as well as tissue factor-mediated activation of FVII.[5,6] Second, slow blood flow (e.g. due to poor vein selection) within the anticoagulant-free plastic tubing can also lead to contact activation of blood coagulation.[7] Third, over-filling of bags and/or under-mixing of blood within the bag can also undoubtedly predispose to clot formation.[8,9] Fourth, inadequate skin cleansing can result in contamination with bacteria, which can proliferate, trigger coagulation and eventually cause clot formation in blood bags.[3,4] High levels of quality control in phlebotomy and blood collection are obviously required to avoid triggering any or all of the aforementioned coagulation activation mechanisms during blood donation.[3,4] Therefore, when blood is properly collected with strict quality control protocols, the risk of clot formation will be very low. Nonetheless, quality control dictates that all donated blood bags must be visually inspected for the presence of clots, and blood bags with visually detectable clots should not be released for transfusion.[4] However, blood clots are often not large enough to be detected by visual inspection.[4] We reckon that the incidence of clots in donated blood bags is low in developed countries due to a high level of quality control and haemovigilance, and the use of automated blood collection monitors.[10] In developed countries, clots in donated blood bags are generally reported as rare and are only occasionally encountered during leucocyte depletion procedures (in the component laboratory) or during transfusion (at the bed side).[4] In contradistinction, the incidence of blood clots would be expected to be high in developing countries (including Nigeria) due to a low level of quality control, poor haemovigilance and lack of automated blood collection monitors.[11,12] Clots in donated blood bags usually block transfusion filters and obstruct blood flow. The effect of a clot on the rate of transfusion blood flow, can be predicted from the size

of the clot. Partially obstructive clots (which are relatively small in size) would slow down the rate of transfusion undesirably. Completely obstructive clots (which are relatively large in size) would shut down the transfusion. Therefore, blood bags that contain partially or completely obstructive clots are usually returned to the blood bank for documentation and replacement with other compatible blood bags.

The incidence, risk factors and root-cause analysis of clotted blood bags have not been adequately studied in Nigeria. Nonetheless, a recent and preliminary study revealed that the incidence of clotting in blood bags in northwest Nigeria was quite high at about 3%[13], and was attributed to poor quality control within the blood transfusion service.[1,11,12] Moreover, the study[13] revealed that the risk of clotting was significantly affected by variations in donor ABO blood groups due to their physiological influence on blood coagulability.[14] Hence, the study had demonstrated that non-O blood groups (that are naturally endowed with 'physiological hypercoagulability')[14,15] were associated with increased risk of clotting, while group-O (that is naturally endowed with physiological hypocoagulability)[14,16] was associated with reduced risk of clotting in blood bags.[13]

Haemoglobin-S (Hb S) is a variant of Hb A that arises as a result of GAG>GTG base transition at codon-6 of the β-globin gene on chromosome-11, which leads to substitution of glutamic acid (polar hydrophilic amino acid) by valine (neutral hydrophobic amino acid) in position-6 of the β-globin chain (βGlu6Val).[17,18] Consequently, HbS has less anionic potential, slower electrophoretic mobility and reduced deoxygenated solubility that leads to polymerisation and red cell sickling.[17,18] Prevalence of sickle β-gene trait in Nigeria and other tropical African countries is as high as 25-30%.[19] This is because the sickle cell trait (SCT) protects against severe malaria and confers survival advantages through natural selection,[20] balanced polymorphism,[21] and immuno-biochemical protective mechanisms against malaria parasites.[22] People with SCT are genetically heterozygous for the sickle β-globin gene and their red cells have the Hb AS genotype expressing both Hb S (20-40%) and Hb A (60-80%).[17,23] The abundance of Hb A prevents sickling under physiological conditions, hence the red cell life span is normal in SCT and affected individuals are symptomless and have a normal life expectancy.[24] Nonetheless, the SCT confers upon its carriers a significant state of hypercoagulability,[25] which results in an undesirably elevated risk of venous thromboembolism among apparently healthy carriers of the trait.[26] The SCT-associated hypercoagulability has been pathophysiologically attributed to the effect of sub-clinical red cell sickling,[27] which increases red cell rigidity,[28] raises blood viscosity,[28] scrambles red cell membrane phospholipids,[29] releases procoagulant phospholipids,[30] and increases the activation rate of clotting factors.[27,30] Moreover, SCT has also been associated with increased expression of monocyte-derived tissue factor, which undesirably aggravates the background hypercoagulability in addition to sub-clinical red cell sickling.[27] It has also been reported that sickling of SCT red cells occurs in the relatively hypoxic environment within donated blood bags; a situation that is thought to be responsible for the high incidence of filter-clogging during leuco-depletion procedures on whole blood units donated by persons with SCT.[31] Nonetheless, the World Health Organization (WHO) and blood transfusion centres worldwide do not consider SCT as a contraindication for blood donation as long as the blood is not subjected to leuco-depletion or used for transfusing foetuses (intra-uterine), neonates and patients with sickle cell disease.[32] Therefore, many apparently healthy persons with SCT constitute a significant proportion of eligible blood donors in tropical countries such as Nigeria.[33]

We therefore reckon that SCT is haemo-rheologically associated with 'pathological hypercoagulabilty',[25-30] hence SCT may predictably increase the risk of clotting in donated blood bags. To the best of our knowledge, the relationship between donor SCT and clotting in blood bags has not been previously studied. Hence, we present a dual working hypothesis. First, we hypothesise that SCT (which is associated with pathological hypercoagulability)[25-30] would be independently associated with a high risk of clotting in blood bags. Second, we hypothesise that donor SCT would act synergistically with non-O blood groups (which are endowed with physiological hypercoagulability)[14,15] in escalating the risk of clotting, thus the coinheritance of both features (SCT and non-O blood groups) in the donor would predictably result in higher risk of clotting in blood bags. If our hypotheses are correct, blood bags with clots will have significantly higher relative frequencies of SCT and non-O blood groups in comparison with control blood bags. In order to test our hypotheses, we conducted an analysis of the pattern of haemoglobin phenotypes and ABO blood groups of clotted blood bags in Nigeria. The aim of the study is four-fold. First, to determine the frequencies of Hb phenotypes and ABO blood groups of clotted blood bags. Second, to determine the risks of clotting in blood bags that are associated with SCT, independently of and in combination with, non-O blood groups. Third, to elucidate the economic and clinical implications of clots in blood bags with respect to the practice of transfusion medicine in Nigeria and other low resource tropical African countries. Fourth, to proffer a practicable strategy that may possibly mitigate SCT-associated risk of clotting in blood bags in Nigeria, and indeed other African countries with high prevalence of SCT.

MATERIALS AND METHODS

Blood bank setting and study description

Similar to other low-resource and under-developed countries, blood donation procedures in Nigerian blood banks are entirely manual without any automation within the context of sub-optimal quality control and poor haemovigilance,[1,11,12] which predispose to high incidence of clotting.[13] Blood bags that contain completely or partially obstructive clots are usually returned to blood banks where they are replaced with newly cross-matched compatible blood bags. The number and blood groups of the clotted blood bags are documented in log books before they are eventually discarded by the blood bank personnel. Regrettably, root-cause analysis of blood clots in donated blood bags are not routinely carried out by Nigerian blood banks due to the aforementioned poor quality control and deficient haemovigilance practices.[1,11,12] With the largest back population of over 200 million, SCT frequency of 25-30% and SCD prevalence of 1-3%, Nigeria carries the heaviest burden of the sickle cell gene in the world.[34] Nonetheless, Nigerian blood banks do not routinely screen prospective donors for SCT, despite WHO recommendation that urges countries with high prevalence of sickle cell gene to screen their donors.[2] This study is an analysis of the frequencies and clotting risks of SCT (and its interaction with non-O blood groups) as seen in pre-discarded clotted blood bags accrued from three tertiary hospitals in north western Nigeria during 2017.

Data generation, retrieval, and collation

This is a prospective study of pre-discarded clotted blood bags accrued from three tertiary hospitals in northwest Nigeria: Rasheed Shekoni Teaching Hospital, Dutse; Murtala Muhammed Specialist Hospital, Kano; and Aminu Kano Teaching Hospital, Kano. Pre-discarded clotted blood bags (of whole blood and packed red cells) were consecutively captured, with due approval of the ethics committee of Rasheed Shekoni Teaching Hospital, from the blood banks of the designated hospitals during 2017 until the data from 100 bags were collated. An equal number of randomly selected un-clotted blood bags (i.e., blood bags without clots) were used as controls. The donor age, sex and ABO blood group of clotted and control blood bags were retrieved from the designated hospitals' blood bank records. However, the haemoglobin (Hb) phenotypes of the clotted and control blood bags were prospectively determined since Hb phenotyping, despite the high prevalence of SCT in the local donor population, is not routinely conducted and documented in Nigerian blood banks.[33]

Determination of donor ABO blood groups

ABO blood groups of donors of blood bags (clotted and control) were determined at the time of donation by standard manual techniques of cell (forward) grouping using monoclonal anti-A and anti-B against donors' red cells in saline tubes at room temperature, and read for agglutination after 15-minute incubation. Donor red cell groups were confirmed by reverse (serum) grouping using donor sera against standard A and B red cells. On the basis of the pattern of agglutination, donor red cells were categorised as group O, A, B or AB.[35]

Determination of donor Hb phenotypes

Hb phenotypes of donors of blood bags (clotted and control) were determined by Hb electrophoresis at a pH of 8.6 on cellulose acetate paper. On the basis of electrophoretic patterns, the Hb phenotypes were categorised as normal (Hb AA) or SCT (Hb AS).[36]

Calculations and statistical analysis

Data accrued from the designated hospitals were collated. Frequencies of Hb phenotypes (AA or AS) and ABO blood groups among the study cohort were determined as percentages. Donor age of the study cohort was calculated in years and presented as mean and standard deviation, while sex profile of the study cohort was presented as percentages of male and female donors. Values of the aforementioned parameters were compared between clotted and control blood bags by using the Student t-test for mean values and X^2 -test for percentages, and p-values of less than 0.05 were considered statistically significant.

The risk of clotting in blood bags were determined as odds ratios (OR) for 3 risk categories viz: Category-1: 'SCT (Hb AS) and blood group-O', Category-2: 'Hb AA and non-O blood groups, and Category-3: 'SCT (HbAS) and non-O blood groups'. Odds ratios for each category were determined by case-control logistic regression analysis using the following inputs:

***Risk Category-1*:** The OR of clotting due to presence of 'SCT (Hb AS) and blood group-O'= *(no. of clotted blood bags with SCT and blood group-O ÷ no. of clotted blood bags with Hb AA and non-O blood group)/ (no. of control bags with SCT and blood group-O÷ no. of control bags with Hb AA and non-O blood group).* This category serves as the test category that determines the OR for SCT as an 'independent' risk factor for clotting in blood bags since blood group -O is not associated with any hypercoagulability.

***Risk Category-2*:** The OR of clotting due to presence of HbAA and non-O blood groups'= *(no. of clotted blood bags with HbAA and non -O blood groups ÷ no. of clotted blood bags with SCT (Hb AS) and blood group-O)/ (no. of control bags with HbAA and non-O blood groups ÷ no. of control bags with SCT (Hb AS) and blood group-O).* This category serves as the test category that determines the OR for non-O blood groups as 'independent' risk factors for clotting in blood bags since HbAA is not associated with any hypercoagulability.

***Risk Category-3*:** The OR of clotting due to presence of 'SCT (HbAS) and non-O blood groups' = *(no. of clotted blood bags with SCT and non-O blood groups ÷ no. of clotted blood bags with HbAA and blood group-O)/(no. of control bags with SCT and non-O blood groups ÷ no. of control bags with HbAA and blood group-O).* This

category serves as the test category that determines the OR for the 'synergistic' hypercoagulable effects of SCT and non-O blood groups as combined risk factors for clotting in blood bags.

An 'increased risk OR' (i.e. OR>1) was considered to be statistically significant if the lower limit of the CI95% was greater than 1.0 with P<0.05. An OR is considered to be statistically insignificant if the range of its CI95% included 1.0 with P>0.05. Statistical analyses were performed using computer software SPSS version 15.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Distribution of donor age, sex and relative frequencies of Hb phenotypes and ABO blood groups among clotted and control bags are shown in Table 1. There were no significant differences between clotted and control blood bags with respect to donors mean ages (29.3 vs. 27.8, p>0.05) and proportions of male (97% vs. 96%, p>0.05) and female (3% vs. 4%, p>0.05) blood donors. However, the relative frequencies of Hb phenotypes and ABO blood groups revealed that in comparison with control blood bags, clotted blood bags had significantly higher relative frequencies of SCT [HbAS] (47% vs. 24%, p<0.05) and non-O blood groups (69% vs. 46%, p<0.05), with corresponding lower frequencies of HbAA (53% vs. 76%, p<0.05) and blood group-O (31% vs. 54%, p<0.05). The odds ratio (OR) values for the risk of clotting in blood bags in three risk categories is shown in Table 2. Category-1:'SCT (Hb AS) and blood group-O' had an elevated OR value of 1.89 ($CI_{95\%}$:1.43-2.34, p=0.031); category-2: 'HbAA and non-O blood groups' had a comparable elevated OR value of 1.91 ($CI_{95\%}$:1.44-2.55, p=0.022); while category-3: 'SCT (Hb AS) and non-O blood groups' had the highest OR value of 2.97 ($CI_{95\%}$:2.55-3.45, p=0.015).

DISCUSSION

Blood donor panels in Nigeria are predominated by young people as shown by the donor mean ages of less than 30 years found in this study. This finding is consistent with the demographic characteristics of Nigerian blood donors as reported in previous studies,[37] and is a reflection of the demographic structure of Nigeria as a developing country with a relatively young population in comparison to the developed countries.[38] Moreover, younger people are relatively more educated, and are therefore more amenable to donor recruitment campaigns.[38] The preponderance of male donors found in this study is interpreted to be a reflection of the general low level of blood donation among the female population in Nigeria.[39] Despite the fact that blood donation is acceptable from healthy females that are not pregnant or breast-feeding,[2] there is a misconception in the general Nigerian population that women are not eligible to donate blood.[39]

Table 1: Distribution of donor age, sex and relative frequencies of Hb phenotypes and ABO blood groups among clotted and control bags

Parameters	Clotted blood bags (n=100)	Control blood bags (n=100)	p-value
Age of donors [years] (Mean ± SD)	29.3±3.3	27.8±2.5	p>0.05
Number and proportion (%) of male donors	97(97)	96(96)	p>0.05
Number and proportion (%) of female donors	3(3)	4(4)	p>0.05
Number and proportion (%) of bags with normal Hb phenotype [HbAA]	53(53)	76(76)	P<0.05
Number and proportion (%) of bags with sickle cell trait (SCT) [HbAS]	47(47)	24(24)	P<0.05
Number and proportion (%) of bags with blood group-O	31(31)	54(54)	P<0.05
Number and proportion (%) of bags with non-O blood groups [A+B+AB]	69(69)	46(46)	P<0.05

Table 2: Odds ratio (OR) values for three risk categories with respect to interactions between Hb phenotypes and ABO blood groups

Risk Categories	Clotted bags (n=100)	Control bags (n=100)	Odd Ratio	Inference
SCT [Hb AS] and blood group-O: No. of bags (%)	29(29)	18(18)	1.89 ($CI_{95\%}$:1.43-2.34, p=0.031)	SCT is independent risk factor for clotting in blood bags
HbAA and non-O blood groups: No. of bags (%)	38(38)	24(24)	1.91 ($CI_{95\%}$:1.44-2.55, p=0.022)	Non-O groups are independent risk factors for clotting in blood bags
SCT [Hb AS] and non-O blood groups: No. of bags (%)	46(46)	22(22)	2.97 ($CI_{95\%}$:2.55-3.45, p=0.015)	SCT and non-O groups synergistically increase risk of clotting in blood bags

This misconception must be rectified by re-configuring our donor mobilisation strategy in order to target and sensitise the female sector,[40] which constitutes about half of the Nigerian population,[38] towards voluntary blood donation. Lack of significant differences in donor age and sex distribution between clotted and control blood bags suggested that age and sex did not affect the risk of clot formation in the blood bags studied in this research, although the strength of this observation may be limited by the very small number of female donors. Nonetheless, there are striking and significant differences between clotted and control bags with respect to the distribution of Hb phenotypes and ABO blood groups of the donors.

The relative proportions of Hb phenotypes among our control blood bags revealed that SCT occurred with a frequency of 24%, which is consistent with the prevalence of SCT within the general population in Nigeria.[34] Moreover, the relative frequencies of ABO blood groups among the control blood bags reflected the normal distribution in Nigeria wherein group-O occurs with a predominant frequency of greater than 50%,[41,42] which is consistent with a selection pressure imposed by *Plasmodium falciparum* in favour of group-O individuals in malaria endemic regions such as Nigeria.[43] In contrast, blood bags with clots showed a distorted distribution of Hb phenotypes and ABO blood groups with significant excess in the relative frequencies of SCT (47%) and non-O blood groups (69%). These findings reaffirmed the prothrombotic potentials of both SCT[25,27] and non-O blood groups,[15] which are consistent with our working hypotheses.

The observed variations in ORs for risks of clotting among the three categories of blood bags in this study can be interpreted with respect to coagulability profiles of Hb phenotypes and/or ABO blood groups. Category-1 bags (SCT and blood group-O; OR: 1.89) and category-2 bags (HbAA and non-O blood groups; OR: 1.91) had comparable OR values. These two OR values (1.89 and 1.91) suggest that SCT and non-O blood groups are independently associated with comparatively similar but modestly elevated clotting risks, and that blood bags with SCT or non-O blood groups were about 2 times (ORs: 1.89 and 1.91) more likely to develop blood clots than control bags, which is consistent with the separate hypercoagulable effects of SCT[25,27] and non-O blood groups.[15] However, category-3 bags (SCT and non-O blood groups) had the highest OR value of 2.97, which suggests that blood bags with SCT and non-O blood groups were about 3 times (OR: 2.97) more likely to develop blood clots than control bags. This finding is interpreted to be a manifestation of the combined (synergistic) hypercoagulability profiles of both SCT[25,27] and non-O blood groups.[15] The pattern of OR values obtained among the three risk categories in this study is therefore in agreement with our research hypotheses, which proposed that SCT is an independent risk factor for clotting in blood bags, and that SCT would act synergistically in combination with non-O blood groups in escalating the risk of clotting in blood bags. This synergistic thrombotic effect is due to the fact that on the one hand, non-O blood groups are associated with elevated levels of clotting factors (vWF and FVIII),[14] while on the other hand, SCT is associated with increased clotting factor activation potential.[25,27,30] This synergism can be viewed as an inter-play between 'abundance of fuel' (non-O group effect) and 'high ignition potential' (SCT effect). Thus, blood bags donated by persons who coinherited both features (SCT and non-O blood groups) were associated with a clotting risk (OR: 2.97) that was higher than the risks associated with blood bags donated by individuals who inherited SCT (OR: 1.89) or non-O blood groups (OR: 1.91) separately. Interestingly, this 'in-vitro' prothrombotic synergism between SCT and non-O blood groups as observed in blood bags in this study had in fact been previously reported in-vivo among patients with clinical diagnoses of deep vein thrombosis in Nigeria.[44]

Root-cause analysis for clotted blood bags (which is neither routinely carried out nor documented in Nigerian blood banks) is outside the scope of this study because of the lack of detailed procedural and haemovigilance data within the blood bank records of the study hospitals. However, the findings of this study suggest that irrespective of the root-cause or trigger mechanisms for clotting (as elucidated in 'introduction') within the blood bags studied in this report, SCT is independently and synergistically (in conjunction with non-O blood groups) an important risk factor for the development of clots in donated blood bags in Nigeria. Nonetheless, we reckon that the clotting risk associated with SCT is undoubtedly perpetuated by the prevailing level of poor quality control protocols within the Nigerian blood transfusion service.[1,11,12] It must therefore be appreciated that frequent occurrence of clots in blood bags donated by persons with SCT would have dual adverse implications (economic and clinical) on the practice of transfusion medicine in low resource tropical settings such as Nigeria where up to one quarter of the general population and eligible donor population carry the SCT.[33,34]

The economic implications arise from the fact that all clotted blood bags (whole blood or packed cells) are eventually discarded, and all haemostatic components (such as fresh frozen plasma, cryoprecipitate and platelets concentrates) that were derived from any clotted whole blood bags are considered qualitatively defective and must be traced, retrieved and discarded.[45] The financial losses associated with discarding clotted blood products are substantial and undesirable in a low resource tropical country such as Nigeria where health services are already grossly underfunded.[46] The clinical implications arise from the fact that discarding clotted blood products in Nigeria would invariably worsen pre-existing perennial donor blood inadequacy[47] resulting from the effects of strong donor inertia,[48] poor female gender participation in blood donation,[39,40] seasonal donor shortfalls due to agrarian activities,[49] high rate of donor rejection due to endemic transfusion transmissible infections,[50] and high rate of donor deferral due to a myriad of asymptomatic anaemias caused by poor diet,[51,52] endemic tropical parasitic infestations[53] or excessive

donations among commercial donors (who constitute a significant proportion of donor panels in Nigeria).[54] Thus, frequent clotting and wastage of donated blood in Nigeria would only make it more difficult to offset the high transfusion demand for maternal and childhood anaemia as well as anaemias resulting from poverty, malnutrition, haemoglobinopathies, and endemic tropical diseases such as malaria, tuberculosis and HIV/AIDS.[34,38,55,56] Finding immediate replacements for clotted blood bags is often difficult in developing tropical countries due to scarcity of donors, and lack of efficient and organised transfusion services.[11,47,48] The difficulty is even severer if clotting affects bags of red cells with rare phenotypes such as the RhD negative phenotype.[57] Moreover, if clotting affects autologous blood bags,[58] it may necessitate the use of allogeneic blood, which would potentially expose the recipients to immunological and infective risks of allogeneic transfusion. Therefore, every effort must be made to minimise the risk of clotting in blood bags and its undesirable consequences.

We strongly believe that the risk of SCT-associated clotting and its attendant economic and clinical implications can be minimised by improving the level of quality control and introducing standardised root-cause analysis for clotted blood bags in the Nigerian national transfusion service. Nonetheless, it has previously been demonstrated that SCT is haemo-rheologically associated with hyperviscosity (both at rest and during exercise),[59] which would undoubtedly aggravate SCT-associated hypercoagulability.[25-30] This can easily be inferred from the well-established haemo-rheological relationship between hyperviscosity and thrombotic clotting risk.[60] Moreover, hydration studies have suggested that SCT-associated hyperviscosity, whether during exercise or at rest (as is the case during blood donation), can simply be preemptively ameliorated by optimum oral hydration with water.[59] In view of the aforementioned haemo-rheological relationship between blood viscosity and hydration in SCT, we present a twin recommendation to help mitigate the risk of clotting in blood bags donated by persons with SCT. First, we recommend routine donor screening for SCT in Nigeria (and indeed other tropical regions in which the SCT is prevalent), as already advocated by the WHO[2] and recommended by local researchers.[33] A simple and cheap HbS solubility test (rather than more costly Hb electrophoresis) would suffice as an effective and rapid SCT screening test in low resource tropical countries.[36] Second, we recommend that blood banks and donation centres in Nigeria (and indeed other tropical regions in which the SCT is prevalent) should consider the possible application of a pre-donation hydration strategy for SCT-positive donors. The rationale behind our recommendations for pre-donation hydration for SCT-positive donors emanates from the fact that haemo-rheological studies suggest that reduction in blood viscosity decreases the risk of clotting within blood vessels (in-vivo)[60], and we believe that this fact would equally be true within donated blood bags (in-vitro), if the donor is pre-hydrated. Hence, pre-donation hydration could predictably reduce SCT-associated hyperviscosity,[28] which could consequently down-regulate hypercoagulability and presumably alleviate in-vitro clotting risk within blood bags donated by pre-hydrated SCT-positive donors. Furthermore, this strategy would also provide an additional benefit (bonus) of minimizing the risk of donation-associated syncope, the incidence of which had previously been shown to be mitigated by pre-donation hydration.[61] If we borrow from the work of France et al (2010)[61], pre-hydration strategy would be quite cheap for the blood bank (using plain water) and convenient for the donor (oral administration). In practical terms about 500mL of water is consumed prior to start of donation, and at least 30 minutes should elapse between water consumption and start of donation[61]; this is necessary to allow sufficient time for water absorption in order to achieve significant and desirable reduction in blood viscosity of donors with SCT.

This study has two limitations. First, donor Hb phenotypes were determined solely by alkaline electrophoresis, which cannot distinguish Hb S from rare Hb variants such as Hb D, Hb G and Hb Lepore that can only be distinguished from one another using additional tests such acid gel electrophoresis or high performance liquid chromatography; these additional tests were not available at the hospitals in which this study was conducted. Second, the risk of clotting in blood bags donated by persons with Hb AA and blood group-O was not calculated. That was because the main thrust of our study was on the hypercoagulability profiles of SCT and non-O blood groups; and neither Hb AA nor blood group-O is associated with hypercoagulability. Hence coinheritance of Hb AA and blood group-O would presumably be associated with very low risk of clotting in blood bags.

CONCLUSION AND RECOMMENDATIONS

Donor SCT is an independent risk factor for clotting in blood bags, and SCT interacts synergistically with non-O blood groups in escalating the prothrombotic risk of clotting in blood bags. Hence, the coinheritance of SCT and a non-O blood group in Nigerian blood donors is associated with higher risk of clotting in blood bags. Clotted blood bags are eventually discarded, resulting in undesirable economic and clinical implications in a low-resource tropical developing country such as Nigeria, where the health budget is inadequate and voluntary donor blood is scarce. We believe that the clotting risk associated with SCT is perpetuated by inadequate quality control. We therefore recommend that the Nigerian blood transfusion service should improve its level of quality control with respect to SCT and the risk of clotting in donated blood bags. Moreover, in view of the fact that SCT-associated hyperviscosity and hypercoagulability can be ameliorated by optimum hydration, we recommend that blood banks and donation centres in Nigeria should routinely screen for SCT, and consider the feasibility of applying a

pre-donation oral hydration strategy for SCT-positive donors. However, there is the need to corroborate the validity and potential benefits of the findings and recommendations of this preliminary study by conducting larger and wider studies to assess the actual extent to which SCT contributes to clotting and wastage of donated blood vis-à-vis the possible need for preemptive pre-donation hydration for eligible donors with SCT in Nigeria, and indeed in other tropical countries in which SCT is prevalent.

REFERENCES

1. Aneke JC, Okocha CE. Blood transfusion safety; current status and challenges in Nigeria. Asian J Transfus Sci 2017;11:1–5. DOI:10.4103/0973-6247.200781.
2. World Health Organization. Blood donor selection: guidelines on assessing donor suitability for blood donation. Geneva 2012. https://apps.who.int/iris/bitstream/handle/10665/76724/9789241548519_eng.pdf. [Accessed: July 10, 2020].
3. World Health Organization. Guidelines on drawing blood: best practices in phlebotomy. Geneva 2010. https://www.ncbi.nlm.nih.gov/books [Accessed: July 10, 2020].
4. Kakaiya R, Aronson CA, Julleis J. Whole blood collection and component processing at blood collection centers. In: Roback JD, editor. Technical Manual. 17th ed. Bethesda: AABB; 2011. pp.187–226.
5. Yun SH, Sim EH, Goh RY, Park JI, Han JY. Platelet activation: the mechanisms and potential biomarkers. Biomed Res Int 2016;2016:9060143. DOI:10.1155/2016/9060143.
6. Naudin C, Burillo E, Blankenberg S, Butler L, Renné T. Factor XII contact activation. Semin Thromb Hemost 2017;43:814-826. DOI:10.1055/s-0036-1598003.
7. Shastry S, Das S. Root-cause analysis for clot in blood bag. Asian J Transfus Sci 2016;10:3-4. DOI:10.4103/0973-6247.175385.
8. Arora D, Dara RC, Raina V, Rawat G, Tiwari AK. CAPA analysis of clotted red cell unit detected during leukodepletion process: importance of quality check on blood collection monitors. Asian J Transfus Sci 2015;9:217-218. DOI:10.4103/0973-6247.162732.
9. Hoque A, Afroz T, Biswas DA. Evaluation of blood clot in the blood bag: experience from Bangladesh. J Med Sci Clin Res 2019;7:327-330. DOI:10.18535/jmscr/v7i6.56.
10. Gupte SC. Automation in blood centres: its impact on blood safety. Asian J Transfus Sci 2015;9:S6-S10. DOI:10.4103/0973-6247.157016.
11. Roberts DJ, Field S, Delaney M, Bates I. Problems and approaches for blood transfusion in the developing Countries. Hematol Oncol Clin North Am 2016;30:477–495. DOI:10.1016/j.hoc.2015.11.011.
12. Aneke JC, Ezeama N, Okocha CE, Onyeyili AN, Ona CE, Ibeh NC, et al. Knowledge, attitude and practice of haemovigilance among healthcare professionals in a Nigerian Tertiary Hospital. Egypt J Haematol 2017;42:108-16. DOI:10.4103/ejh.ejh_25_17.
13. Ibrahim Z, Kani KM, Habib A, Ibrahim UA, Ahmed SG. Clots in blood bags in northwest Nigeria: incidence, correlation with donor ABO blood groups, and implications on blood transfusion in a low resource tropical setting. Trop J Health Sci 2020;27:1-7. Available at: https://www.ajol.info/index.php/tjhc/issue/view/19036.
14. Song J, Chen F, Campos M, Bolgiano D, Houck K, Chambless LE, et al. Quantitative influence of ABO blood groups on factor VIII and its ratio to von Willebrand factor, novel observations from an ARIC study of 11,673 subjects. PLoS One 2015;10:e0132626. DOI:10.1371/journal.pone.0132626.
15. Dentali F, Sironi AP, Ageno W, Turato S, Bonfanti C, Frattini F, et al. Non-O blood type is the commonest genetic risk factor for VTE: results from a meta analysis of the literature. Semin Thromb Hemost 2012;38:535-48. DOI:10.1055/s-0032-1315758.
16. Franchini M, Crestani S, Rossi C, Frattini F, Mengoli C, Giacomini I, et al. O blood group and the risk of major bleeding: a single centre survey. J Thromb Thrombolysis 2013;35:65-66. DOI:10.1007/s11239-0783-x.
17. Flint J, Harding RM, Boyce AJ, Clegg JB. The population genetics of the haemoglobinopathies. Bailliere's Clin Haematol 1993;6:215-222.DOI:10.1016/S0950-3536(05)80071-X.
18. Kaul DK, Fabry ME, Nagel RI. The pathophysiology of vascular obstruction in the sickle cell syndromes. Blood Rev 1996;10:29-44. DOI:10.1016/S0268-960X(96)90018-1.
19. Fleming AF, Storey J, Molineaux L, Iroko EA, Attai EDE. Abnormal haemoglobins in the Sudan savanna of Nigeria: I. Prevalence of haemoglobin-S and relationships between sickle cell trait, malaria and survival. Ann Trop Med Parasitol 1979;73:161-72. DOI:10.1080/00034983.1979.11687243.
20. Elguero E, Délicat-Loembet LM, Rougeron V, Amathau C, Roche B, Becquart P, et al. Malaria continues to select for sickle cell trait in Central Africa. Proc Natl Acad Sci 2015;112:7051-4. DOI:10.1073/pnas.1505665112.
21. Olatunji PO. Malaria and the Sickle Gene: Polymorphism balanced in favour of eradication. Ann Health Res 2018;4:88-96. DOI:10.30442/ahr.0402-1-12.
22. Gong L, Parikh S, Rosenthal PJ, Greenhouse B. Biochemical and immunological mechanisms by which sickle cell trait protects against malaria. Malar J 2013;12:317-317. DOI:10.1186/1475-2875-12-317.
23. Ahmed SG, Ibrahim UA. Haemoglobin-S in sickle cell trait with papillary necrosis. Br J Haematol 2006;135:415-6. DOI: 10.1111/j.1365-2141.2006.06318.x.
24. Barbedo MMR, McCurdy PR. Red cell life span in sickle cell trait. Acta Haematol 1974;15:339-42.
25. Lawrie AS, Pizzey A, Trompeter S, Meiselman, H, Mohandas, N, Dumanski JP, et al. Procoagulant activity in patients with sickle cell trait. Blood Coagul Fibrinolysis 2012;23:268-270. DOI:10.1097/MBC.0b013e32835187f8.
26. Folsom AR, Tang W, Roetker NS, Kshirsangar AV, Derebail AK, Lutsey PL, et al. Prospective study of sickle cell trait and venous thromboembolism incidence. J Thromb Haemost 2015;13:2-9. DOI:10.1111/jth.12787.
27. Westerman MP, Green D, Gilman-Sachs A, Beaman K, Freels S, Boggio L, et al. Coagulation changes in individuals with sickle cell trait. Am J Hematol 2002;69:89-156.
28. Zheng Y, Mark A. Cachia MA, Ge J, Xu Z, Wang C, et al. Mechanical differences of sickle cell trait and normal red blood cells. Lab Chip 2015;15:3138–3146. DOI:10.1039/c5lc00543d.
29. Tait JF, Gibson D. Measurement of membrane phospholipid asymmetry in normal and sickle cell erythrocytes by means of annexin-V binding. J Lab Clin Med 1994;123:741-8.
30. Westerman MP, Cole ER, Wu K. The effect of spicules obtained from sickle red cells on clotting activity. Br J Haematol 1984;56:557-62.
31. Beard MJ, Cardigan R, Seghatchian J, Krailadsiri P, Williamson LM. Variables determining blockage of WBC-depleting filters by Hb sickle cell trait donations. Transfusion 2004;44:422-30. DOI:10.1111/j.1537-2995.2003.00669.x.
32. World Health Organization. Blood donor selection: guidelines on assessing donor suitability for blood donation. Geneva, 2012. Available at: http://www.who.int/bloodsafety/publications/bts_guideline_donor_suitability/en/. [Accessed: July 10, 2020].
33. Ahmed SG, Hassan AW, Ibrahim UA. The frequency and clinical significance of structural haemoglobin variants in donor blood at university of Maiduguri teaching hospital. Niger J Surg Res 2000;2:127-30. DOI:10.4314/njsr.v2i3.12199.
34. Galadanci N, Wudil BJ, Balogun TM, Ogunrinde GO, Akinsulie A, Hasan-Hanga F, et al. Current sickle cell disease management practices in Nigeria. Int Health 2014;6:23-28. DOI:10.1093/inthealth/iht022.
35. Rowley M, Cantwell C, Milkins C. Laboratory aspects of blood transfusion. In: Bain B, Bates I, Laffan M, editors. Practical Haematology. 12th ed. London: Elsevier; 2017. pp.470-496.
36. Wild B, Bain B. Investigation of abnormal haemoglobins and thalassaemia. In: Bain B, Bates I, Laffan M, editors. Practical Haematology. 12th ed. London: Elsevier; 2017. p.282-311.
37. Ugwu AO, Madu AJ, Efobi CC, Ibegbulam OG. Pattern of blood donation and characteristics of blood donors in Enugu, Southeast Nigeria. Niger J Clin Pract 2018;21:1438-43.
38. National Population Commission. Nigeria Demographic and Health Survey 2018. Abuja, Nigeria. Available at: https://www.dhsprogram.com. [Accessed: July 10, 2020].
39. Erhabor O, Isaac Z, Abdulrahaman Y, Ndakotsu M, Ikhuenbor DB, Aghedo F, et al. Female gender participation in the blood donation process in resource poor settings: case study of Sokoto in north western Nigeria. J Blood Disord Transfus 2013;5:176. DOI:10.4172/2155-9864.1000176.
40. Anyanwu-Yeiya CC, Sonubi O, Kotila TR. Targeting females as voluntary non remunerated donors in developing nations. J Blood Disord Transfus 2015;S4:S4-002. DOI:10.4172/2155-9864.1000S4-002.
41. Chima OK, Mohammed TB, Aisha KG, Alhaji SA, Muhammad BM, Kwaru AH. ABO and rhesus blood groups among blood donors in Kano, North-Western Nigeria. Niger J Basic Clin Sci 2012;9:11-3. DOI:10.4103/0331-8540.102105.
42. Ahmed SG, Obi SO. The Incidence of ABO and Rhesus-D Blood Groups in North-East Nigeria. Niger J Med 1998;7:68-70.
43. Cserti CM, Dzik WH. The ABO blood group system and Plasmodium falciparum malaria. Blood 2007;110:2250-8. DOI:10.1182/blood-2007-03-077602.
44. Ahmed SG, Kagu MB, Ibrahim UA, Bukar AA. Impact of sickle cell trait on the thrombotic risk associated with non-O blood groups in northern Nigeria. Blood Transfus 2015;13:639-43. DOI:10.2450/2015.0335-14.
45. Armstrong B. Blood collection. ISBT Sci Ser 2008;3:123-6.
46. Hafez R. Nigeria health financing system assessment. World Bank Report, 2018. DOI:10.1596/30174.
47. Ahmed SG, Ibrahim UA, Hassan AW. Adequacy and pattern of blood donations in northeast Nigeria: the implications for blood safety. Ann Trop Med Parasitol 2007;101:725-731. DOI:10.1179/136485907X241442.
48. Ahmed SG, Gamas MG, Kagu BM. Declining frequency of blood donation among elites in Maiduguri, Nigeria. Afr J Med Med Sci 2006;35:359-363.
49. Ahmed SG. Impact of seasonal agriculture on blood donations in Kano, northwest Nigeria and its implications on health care. Ann Trop Pathol 2013;4:77-82.
50. Okoroiwu HU, Okafor IM, Asemota EA, Okpokam DC. Seroprevalence of transfusion-transmissible infections (HBV, HCV, syphilis and HIV) among prospective blood donors in a tertiary health care facility in Calabar, Nigeria; an eleven years evaluation. BMC Public Health 2018;18:645. DOI:10.1186/s12889-018-5555-x.
51. Kagu MB, Ahmed SG, Bashir MA, Malah MB, Usoro A, Gimba I, et al. Deferral pattern of voluntary blood donors at the national blood transfusion service, northeast zonal centre, Maiduguri, Nigeria. Afr J Med Med Sci 2010;39:119-125.
52. Ahmed SG, Kagu MB. Morphological classification of anaemia among first-time blood donors in northwest Nigeria and the implication of empirical haematinics therapy. Int J Biomed Health Sci 2011;7:125-129.
53. Ahmed SG, Uraka AJ. Role of intestinal parasites in donor anaemia and deferral in northwest Nigeria. Afr Sanguine 2010;13:8-10.
54. Ahmed SG, Kagu MB, Abjah UAM. Haematological parameters of blood donors in northeast Nigeria and implication on quality of blood products. Afr Sanguine 2010;13:5-8.
55. Ahmed SG, Ibrahim UA, Kagu MB. The burden of HIV and AIDS on blood bank reserve in northeast Nigeria. Trans Roy Soc Trop Med Hyg 2007;101:618-620. DOI:10.1016/j.trstmh.2006.09.008.
56. Ahmed SG, Bukar AA, Jolayemi B. Hematological indices of sickle cell anaemia patients with pulmonary tuberculosis in northern Nigeria. Mediterr J Hematol Infect Dis 2010; 2:e2010014. DOI:10.4084/mjhid.2010.014.
57. Ahmed SG. A strategic approach to the problems of providing RhD negative blood in geographic areas with low RhD negativity: a Nigerian perspective. Transfus Med Rev 2010; 24:140-146. DOI:10.1016/j.tmrv.2009.11.005.
58. Loubser PG. Clot formation in a blood collection bag used for acute normovolemic hemodilution. J Cardiothorac Vasc Anesth 2008; 22:801-802. DOI:10.1053/j.jvca.2008.02.007.
59. Tripette J, Loko G, Samb A, Gogh BD, Sewade E, Seck D, et al. Effects of hydration and dehydration on blood rheology in sickle cell trait carriers during exercise. Am J Physiol Heart Circ Physiol 2010;299:H908-H914. DOI:10.1152/ajpheart.00298.2010.
60. Baskurt OK , Meiselman HJ. Iatrogenic Hyperviscosity and Thrombosis. Semin Thromb Hemost 2012;38:854-64. DOI:10.1055/s-0032-1325616.
61. France CR, Ditto B, Wissel ME, France JL, Dickert T, Rader A, et al. Pre-donation hydration and applied muscle tension combine to reduce presyncopal reactions to blood donation. Transfusion 2010; 50:1257-1264. DOI:10.1111/j.1537-2995.2009.02574.x.