RESEARCH ARTICLE

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Effects of repeated blood donation on iron status of blood donors in Zimbabwe: A cross-sectional study

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Abstract

Introduction: Iron deficiency is a major complication of repeated blood donation. However, most of the blood screening methods employed by blood collection agents do not include iron status markers, leading to possible subclinical iron deficiency. The aim of this study was to evaluate the effects of repeated blood donation on the iron status of this vulnerable population in Zimbabwe.

Methods: All donors were categorized into groups based on number of donations made in the previous 2-year period prior to enrolment into the study. Serum iron, total iron-binding capacity (TIBC), and ferritin were analyzed on automated chemistry analyzers while transferrin saturation (TSAT) was calculated. The Wilcoxon rank-sum and ANOVA tests were used to assess the variation of iron profiles by gender and frequency of donations. All data analysis was performed using Stata software v13.

Results: Study participants included 170 repeat donors and 20 first-time blood donors. The median (IQR) age was 23 (19-27) years, while the majority were males 57% (n = 109/190). The overall prevalence of iron deficiency and reduced iron stores was 12.6% and 38.9%, respectively. There were statistically significant differences between males and females in all the iron status parameters ($P < .05$). TIBC increased with number of donations, while iron, ferritin, and TSAT decreased with increased number of donations.

Conclusion: A high proportion of blood donors had iron deficiency despite being eligible to donate. Repeated blood donation may lead to substantial reduction in iron stores among blood donors. Inclusion of iron biochemical markers may enhance proper screening and monitoring of blood donors in Zimbabwe to prevent development of iron deficiency anemia.

KEYWORDS
blood donation, iron deficiency, iron status, negative iron balance, reduced iron stores

1 | INTRODUCTION

Provision of safe and adequate blood is an integral part of every country's national health care policy. This is made possible through voluntary or paid blood donation.[1] Consequently, the health and well-being of blood donors are of paramount importance in transfusion medicine. Blood donor retention saves blood services institutions time and resources.[1] Efforts to retain donors and get adequate blood for transfusion purposes are hampered by donor deferrals for various reasons: low hemoglobin being the major reason worldwide.[1] Deferrals have cost and health care implications to blood services and a negative effect on the donors to give blood in the future.[1] For this reason, blood services have a responsibility to protect blood donors against developing anemia.[2]

In Zimbabwe, prior to giving blood, donors are screened by the copper sulfate screening method, which is based on the principle of specific gravity.[3] However, this screening test has its own challenges. Firstly, the use of hemoglobin for screening has poor sensitivity in detecting donors with low hemoglobin, especially during the early stages of iron deficiency.[4] Anemia is a late manifestation of iron deficiency, hence the measurement of hemoglobin is a poor surrogate marker for iron depletion.[5] Secondly, the capillary (finger-prick) hemoglobin tends to overestimate venous hemoglobin, especially in iron deficient donors.[5] Some studies have reported that, since the copper sulfate screening test does not measure the actual hemoglobin, it erroneously includes anemic and excludes eligible blood donors.[3,4,6] Iron depletion is the earliest stage of iron deficiency and signifies that iron stores are decreased or absent, but hemoglobin levels may be normal.[4] Hence, it becomes imperative that iron status be assessed among blood donors.

Serum ferritin, TSAT, serum soluble transferrin receptors, and the serum soluble transferrin receptors-ferritin index are more accurate than hemoglobin and classic red cell indices.[7] Ferritin and TSAT show a strong correlation with bone marrow iron stores: the gold standard for measuring iron stores.[8,9]

Previous studies have reported decreased iron stores with repeated blood donation across several regions.[2,4,5,10] Some studies across Africa have reported that iron markers of blood donors do not differ significantly from healthy controls.[11,12] However, in sub-Saharan Africa (including Zimbabwe), previous reports have shown iron overload among the population.[13] Thus, investigating the iron stores of the blood donors is pivotal to guide policy and practice. Evidence on the effect of donation is crucial for repeat blood donors within this population. Hence, we sought to investigate the effect of blood donation on iron status among blood donors in Zimbabwe.

2 | MATERIALS AND METHODS

2.1 | Study design

This was a cross-sectional study to evaluate the effects of repeated blood donation on iron status of randomly selected blood donors who attended the National Blood Services Zimbabwe (NBSZ) blood collection events in August 2016. The study population was all Pledge 25 club members who passed the copper sulfate screening test during men's and ladies gala at NBSZ, Harare, Zimbabwe.

2.2 | Study population

Pledge 25 club is a special nationwide club for voluntary young and healthy citizens who pledge to regularly donate safe units of blood at least 25 times in their lives. Most of the Pledge 25 club members start giving blood during high school till they attain adulthood. The primary aim is to allow for smooth transition from school donors into safe adult donors, thereby effortlessly creating a future pool of informed adult blood donors. Voluntary donors who constituted the Pledge 25 club were first-time donors as well as repeat donors from all parts of the country. This group of donors is key for the provision of blood and blood products in the whole country, but at the same time repeated donation exposes them to potential effects of repeated blood loss, hence making them a vulnerable population.

2.3 | Study setting

National Blood Service Zimbabwe is a nonprofit-making organization mandated by the ministry of Health and Child care to collect, process, and distribute safe, adequate blood and blood products throughout the country. It relies on voluntary non-remunerated blood donation. Blood is obtained mainly from school children aged 16 years and above, all over Zimbabwe accounting for 70% of donations. The NBSZ encourages scholars to continue donating blood after leaving school. For this reason, the NBSZ set up the Pledge 25 club in 1995.

2.4 | Study procedure

Prior to giving blood, every blood donor responded to questions on the history of risk behavior for transfusion transmissible infections (TTIs). Those who passed this stage were screened for anemia using the copper sulfate screening test. Prior to donation, 3 mL of donor's blood samples were collected into plain tubes. Samples were marked by the barcode number and serum was obtained after centrifugation at $900 \times g$ for determination of ferritin, iron, and TIBC. Donor demographics and blood donation history (over the past 2 years prior to enrolment into the study) were obtained from e-Delphyn, the laboratory information system (LIS).

2.5 | Laboratory assessment of iron, ferritin, and total iron-binding capacity

Serum iron and ferritin were analyzed on the Mindray BS 800 (Shenzhen Mindray bio-Medical Electronics, China) chemistry

TABLE 1 Demographic and clinical profiles of blood donors in Zimbabwe

Characteristic	Overall n = 190	Male donors n = 109	Female donors n = 81	P value
Median (IQR) age in years	23 (19-27)	25 (21-29)	20 (18-23)	P < .001*
Median (IQR) serum iron levels (µmol/L)	13.0 (10.0-17.8)	14.4 (11.0-18.8)	12.0 (8.8-16.0)	P < .001*
Median (IQR) serum ferritin levels (µg/L)	30 (18-56)	40 (21-76)	22 (15-40)	P < .001*
Median (IQR) serum TIBC (µmol/L)	74.2 (63.3-82.3)	72.3 (61.7-80.1)	78.1 (65.8-84.3)	P = .011*
Median (IQR) TSAT (%)	17.6 (12.0-26.9)	19.7 (14.6-27.8)	14.5 (10.7-22.9)	P < .001*
Number of donations Median (IQR)	4.0 (3.0-6.0)	5.0 (3.0-7.0)	4.0 (2.0-6.	P < .02*

Abbreviations: IQR, interquartile range; TIBC, total iron-binding capacity; TSAT, transferrin saturation.
*Statistically significant with P < .05.

analyzer as previously described by Meng et al,[14] while TIBC was done on the automated analyzer Beckman Coulter Synchron CX9 PRO (Coulter Corporation, USA). Serum iron was quantified using the photometric principle where the measured absorbance is proportional to the serum iron concentration, while serum ferritin was quantified by the absorbance of the agglutination formed using the particle-enhanced immuno-turbidimetric method. Similarly, TIBC was quantified using the photometric principle where the absorbance of the ferrous iron complex is proportional to the concentration of iron bound to transferrin.

Body iron stores are distributed between hemoglobin, ferritin, and non-blood tissue iron.[11,15] Traditionally, the standard biochemical markers of iron status are serum ferritin, transferrin, iron, TSAT, and of late, soluble transferrin receptor.[16] Serum iron alone is not a good indicator of iron stores. Additionally, it is not a sensitive measure of iron deficiency, partly because of daily fluctuations. Transferrin saturation, calculated from serum iron and TIBC, indicates the percentage of binding sites on transferrin that are occupied by iron and is, therefore, a measure of circulating iron that is immediately available for erythropoiesis. Thus, TSAT is a better marker than iron and TIBC.[15,17]

Plasma ferritin is in equilibrium with body stores, and its concentration declines early in the development of iron deficiency.[8,9,18] Serum ferritin concentrations are sensitive indicators of iron deficiency and can be used to detect preclinical iron deficiency states. Thus, serum ferritin is the best test to evaluate the iron stores.[8] Serum ferritin and TSAT are regarded as the most reliable indicators of iron status.[16] The tests are best interpreted together as a profile to give a holistic view of the iron status in patients.

2.6 | Data interpretation

Transferrin saturation was calculated using the formula: % Transferrin Saturation = (serum iron [µmol/L])/(TIBC [µmol/L]) × 100.[19] Interpretation of the results were based on the reference ranges previously reported by Braunstein (2020): Ferritin: 30 to 300 µg/L, TSAT: 20% to 50%, Iron: 11 to 25 µmoL/L (females), 13 to 27 µmoL/L (males) and TIBC: 45 to 81 µmoL/L.[20] Donors were classified as being iron deficient when serum ferritin was below 15 µg/L.[4,18,19,21-23] They were also classified as having reduced iron stores when their serum ferritin

was in the range of 15 to less than 30 µg/L (8, 15, 17). Donors who fell into these two categories (iron deficiency and reduced iron stores) were considered as having a negative iron balance.

2.7 | Statistical analysis

First-time donors (Group 1) were considered as controls upon which the effects of repeated blood donation were compared. Group 2 was made up of those who had donated twice in the 2-year study period, Groups 3, 4, 5, 6, 7, and 8 are made up of people who have donated three, four, five, six, seven, and eight times, respectively, in the study period. Descriptive statistics were used to summarize the baseline demographic and clinical characteristics of the study participants. The two-sample Wilcoxon rank-sum test and ANOVA test were used to analyze the variation of iron profiles by gender and frequency of donations. Significance levels were set at P = .05. All data analysis was performed using Stata software v13 (Stata Corp). The measured iron parameters were also analyzed in each category to identify trends.

3 | RESULTS

3.1 | Donor characteristics

A total of 190 voluntary whole blood donors were enrolled; over half were males, 57% (n = 109/190). Of the 190; 170 (89%) were repeat blood donors and 20 (11%) were donating blood for the first time. The median (IQR) age of the participants was 23 (19-27) years. There was a statistically significant difference in age between males and females (P < 0.001). The median (IQR) serum iron concentration was 13 (10.0-17.8) µmol/L, with males showing higher median (IQR) serum iron levels compared with females {14.4 (11.0-18.8) vs 12.0 (8.8-16.0) respectively, P < .001}. Additionally, the median (IQR) value of ferritin was 30 (18-56) µg/L, which was significantly higher in males compared with females {40 (21-76) vs 22 (15-40) respectively, P < .001}. The median (IQR) of transferrin saturation was 17.6% (12.0-26.9). There were statistically significant differences between males and females in all the iron status parameters under study (See Table 1).

3.2 | Blood donors' distribution history

Two-year blood donation history prior to enrolment in the study showed a median (IQR) of 4 (3-6) donations. There were statistically significant differences in the number of donations between males and females ($P = .02$) in the period under study. Generally, men had relatively more donations than females as the number of donations increased. No female could possibly donate eight times in a 2-year period as per the NBSZ donor regulations, hence group 8 has no
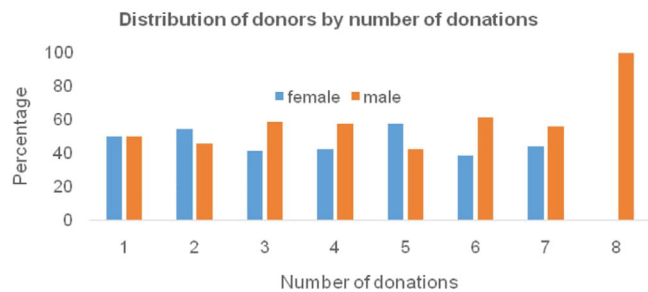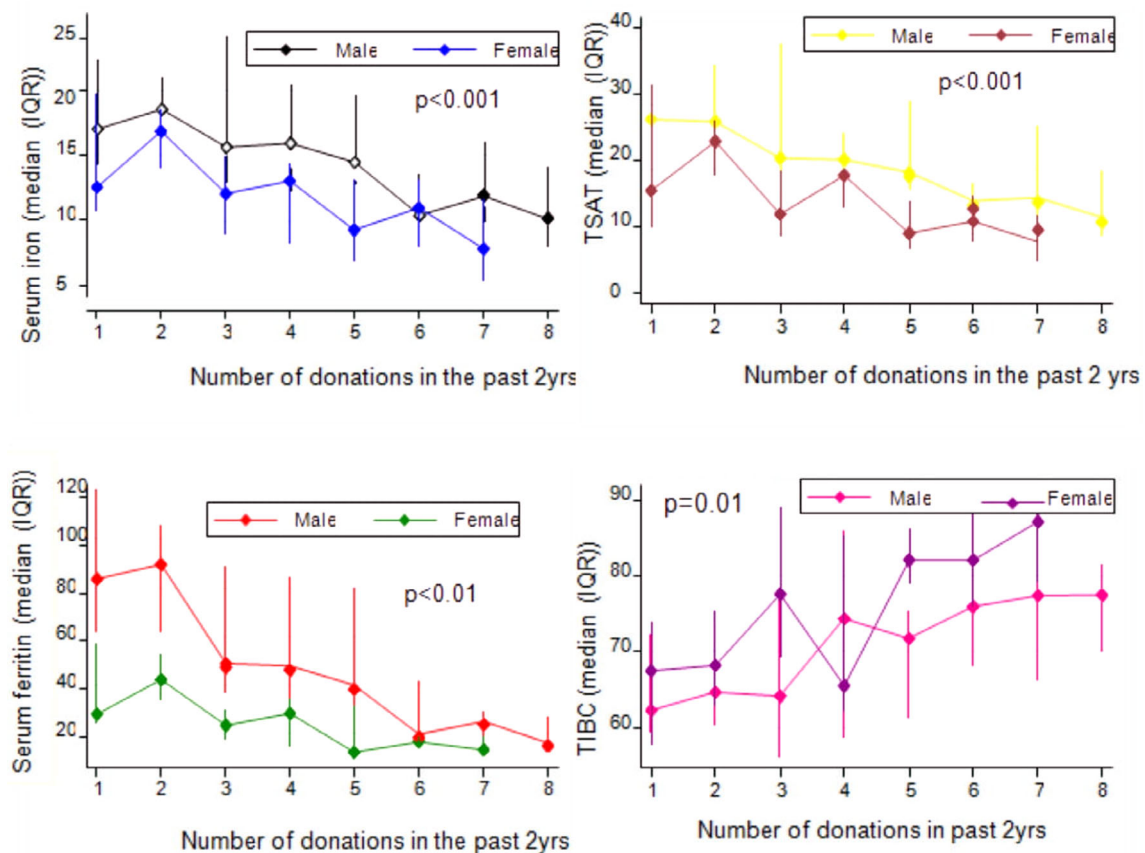

FIGURE 1 Distribution of donors by number of donations and gender

females. However, at enrolment, we have equal number of males and females (control group 1). The blood donors' distribution by gender and donation frequency across the groups is shown in Figure 1.

3.3 | Distribution of the iron status markers by gender and number of donations over the 2 years period

There was a decreased trend in median iron, ferritin and transferrin saturation with increased number of donations (Figure 2). Additionally, we noted a statistically significant difference in male's median iron levels between the control group (group 1) and group 8 (19.73 vs 11.46) ($P = .003$). No statistically significant difference in serum iron levels was observed among females. There were statistically significant differences in mean ferritin levels between group 1 and groups 6, 7, and 8 ($P < .01$) for men, while differences were observed among the controls and groups 6 and 7 ($P < .05$) in females. For TIBC, although the general trends showed an increase in the TIBC concentration with an increased number of donations, there was no statistically significant difference between the control group and the other groups among males. However, among females, there was statistically
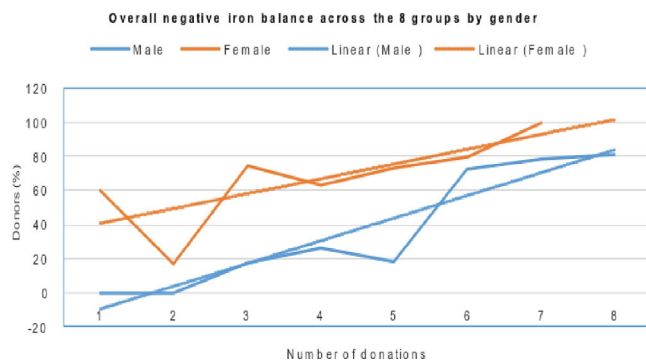

FIGURE 2 Distribution of iron status markers by gender and number of donations over the 2 years period prior to enrolment
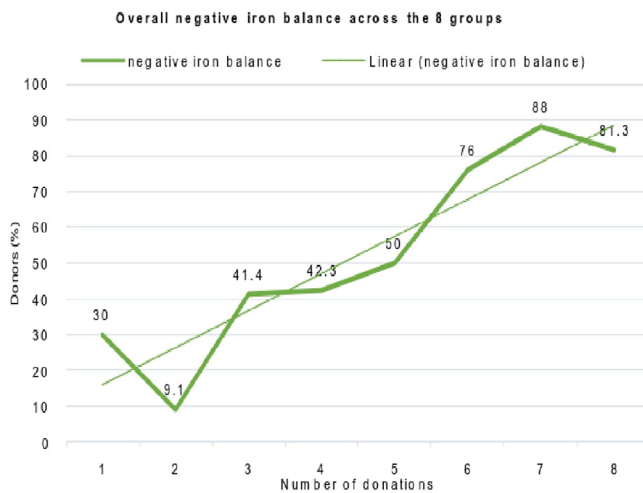
FIGURE 3 Relationship between overall negative iron balance and number of donations over the 2 years period prior to enrolment

FIGURE 4 Distribution of blood donors with an overall negative iron balance over the 2 years period by gender

significant difference between the control group and group 7 only (68.3 vs 83.6 μmol/L) (P = .0043). Conversely, transferrin saturation showed a decreased trend in both gender, with males having a statistically significant difference between group 1 and group 8 (29.0% vs 15.5%) (P < .001). Overall, Figure 2 plots show a general trend of decreased median and interquartile ranges of iron stores markers as the donation frequency increases in the 2-year period under study.

3.4 | Prevalence of negative iron balance (reduced iron stores and iron deficiency) among participants

Overall, the prevalence of iron deficiency and reduced iron stores were seen in the 12.6% and 38.9%, respectively. When these results were compared by gender, females had 22.2% and 44.4% and males 5.5% and 34.9%, respectively. Figures 3 and 4 show the distribution of donors with negative iron balance across the eight groups. Figure 3 shows the overall negative iron balance picture of males and females across the eight groups, and Figure 4 shows the negative iron balances across the eight groups split by gender.

4 | DISCUSSION

Blood banks have a responsibility to protect donors against iron depletion and anemia.[4,19] We conducted this study to evaluate the effects of regular blood donation on iron status of randomly selected blood donors who attended the NBSZ. Our findings confirm the hypothesis that repeat blood donors are more likely to have depleted iron stores than first-time blood donors.

This study showed that iron stores decrease as the number of donations increases, indicating loss of iron during blood donation as previously reported in other studies worldwide.[2,4,10,24] Not surprising, females had significantly lower iron parameters compared with males. This may be attributed to other natural forms of blood losses in females of childbearing age.[11,19,24,25] Our study is consistent with several studies that also reported iron depletions among blood donors.[2,4,19,25]

Different markers for prediction of iron status have been previously reported, including hematological parameters.[11,26,27] Our study is similar to previous studies,[11,25,28] which reported that iron deficiency was prevalent among repeat donors. In a Spanish study, iron deficiency ranged from 21% to 46% for donors who had given between 1 unit and 4 units in the previous year.[25] In a Saudi Arabian study, proportions of 2.2% and 16.3% were reported for iron deficiency and reduced iron stores, respectively.[4] Gaff et al reported 20.5% for iron deficiency among multiple blood donors for males.[10] Generally, there has been a wide range of incidences of iron deficiency in several studies conducted on blood donors.[11,24-27,29] Such differences could be due to different geographical locations, differences in dietary habits, worm infestation, poverty, and policies of the national blood transfusion services.

In addition, different cutoffs and predictive markers used to define reduced or depleted iron stores also have an impact on interpretation, hence different proportions. In our study, we used the WHO-recommended cutoffs of ferritin as a proxy for iron stores.[18,22] We did not include the donors who had failed the copper sulfate screening test, yet this population could have increased the proportions of donors with iron deficiency and reduced iron stores. In a study done in Nigeria, no differences were found in iron parameters between male donors and healthy controls.[12] Donors in the Nigerian study were made up of paid as opposed to voluntary donors in our study, thereby making the study groups' composition dynamics different. In another Nigerian study, regular blood donation had no significant effect on the hemoglobin concentration, packed cell volume, and serum iron levels except for serum ferritin levels, which were significantly reduced.[2]

Blood transfusion services institutions should be focused on maintaining iron balance in blood donors. One of the most frequent observations in long-term blood donation is chronic iron deficiency, which may progress to anemia.[30] Early detection of iron deficiency among blood donors would allow appropriate interventions to be made before a donor is lost due to deferral. The current study findings present a call for the donor screening processes to include markers for iron status such as ferritin in the assessment of the actual donor status for repeat blood donors. Such early detection of iron deficiency among blood donors would allow

appropriate readjustment of donation intervals and would guide the use of iron supplementation. Pre-donation screening and revision of donation intervals are alternative ways of managing the repeat donors in order to prevent the development of iron deficiency among this vulnerable population. Anticipation of the likelihood of losing a donor is very pivotal in donor care and management.

This study has some limitations. First, we did not capture the date of data abstraction and the date of last blood donation. This made it impossible for us to calculate the time since the last donation. Second, we did not assess the effect of possible confounders such as socioeconomic status, diet, or occupation on iron status among blood donors. Third, the data presented here were mainly from Harare, an urban setting, and may not be generalizable to rural parts of the country. Some of the areas are endemic to malaria infection, which invariably affects number of donations and iron stores. Hence, a bigger prospective study with living habits stratification and geographical distribution will add more insights to these initial findings to determine the long-term effects of blood donation.

5 | CONCLUSIONS

A large proportion of blood donors had iron deficiency despite being eligible to donate. Gender differences showed significantly different effects on iron status among voluntary blood donors. An inverse relationship between frequency of blood donation and serum iron, ferritin, and transferrin saturation indicating that repeated blood donation causes a significant reduction in the iron stores among donors in Zimbabwe. Thus, there is need to include biochemical markers for proper screening and monitoring of blood donors to ensure a safer blood donation process for the donors and prevent the development of iron deficiency anemia. Donation intervals may need to be revised and iron supplementation should be considered even for those who are still passing the copper sulfate screening method.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

TRANSPARENCY DECLARATION
The lead author* affirms that this manuscript is an honest, accurate, and transparent account of the study being reported; that no important aspects of the study have been omitted; and that any discrepancies from the study as planned (and, if relevant, registered) have been explained.

AUTHORS' CONTRIBUTIONS
Conceptualization: Donald Vhanda, Vinie Kouamou.

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Funding Acquisition: Donald Vhanda.

Investigation: Donald Vhanda.

Methodology: Donald Vhanda, Collins Timire, Vinie Kouamou.

Project Administration: Donald Vhanda, Frank Chinowaita, Sisodwa Nkomo, Collins Timire, Vinie Kouamou.

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Writing—Original Draft Preparation: Donald Vhanda.

Writing—Review and Editing: Donald Vhanda, Frank Chinowaita, Sisodwa Nkomo, Collins Timire, Vinie Kouamou.

All authors contributed equally in drafting the manuscript, reviewing it critically for intellectual content. The final version for publication was approved by all authors.

The data that support the findings of this study are available from the corresponding author upon reasonable request.

PATIENT OR PUBLIC CONTRIBUTION
Blood donors visiting NBSZ were the study participants. Staff members from NBSZ gave guidance on how to access patient records from the LIS using donor numbers.

DATA AVAILABILITY STATEMENT
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT
The study commenced after approval from the National Blood Service Zimbabwe Research Ethics Committee, Joint Parirenyatwa Hospital and College of Health Sciences Research Ethics Committee (JREC). Study participants were required to sign written informed consent prior to participation in the study.

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