Sparrow Rosemary (Orcid ID: 0000-0002-6689-2983)

Higher donor body mass index is associated with increased hemolysis of red cell

concentrates at 42-days of storage: a retrospective analysis of routine quality control data

Rosemary L Sparrow^{1,2*}, Katherine A Payne^{1,3}, Geoffrey G Adams⁴

¹Formerly Research and Development, Australian Red Cross Blood Service, West Melbourne, Victoria, Australia

²Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Victoria, Australia

³National Manufacturing and Quality Division, Australian Red Cross Lifeblood, Melbourne, Victoria, Australia

⁴Melbourne Dental School, The University of Melbourne, Melbourne, Victoria, Australia

* *Address correspondence to:*

Adjunct Associate Professor Rosemary Sparrow

Transfusion Research Unit, Department of Epidemiology and Preventive Medicine

Monash University, 553 St Kilda Road, Melbourne Victoria 3004 Australia

Tel (mobile): +61 411803326

e-mail: rosemary.sparrow@monash.edu

Running title: Hemolysis and donor factors

Abbreviations: BCRC – buffycoat-poor red cell; BMI – body mass index; CoE – Council of Europe; df – degrees of freedom; FDA – Food and Drug Administration; Hb – hemoglobin; HCT – hematocrit; MVOR - multifactorially adjusted odds ratio; OR – odds ratio; QC – quality control; RBC(s) – red blood cell(s); RCC(s) red cell concentrate(s); WB – whole blood

Funding support: Australian governments fund Australian Red Cross Lifeblood to provide blood, blood products and services to the Australian community.

Disclosures: No conflict of interest to disclose

Figures: 2

Tables: 6

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: [10.1111/trf.16203](https://doi.org/10.1111/trf.16203)

Supplementary documents: 1

Word Count: 3990

ABSTRACT

Background: For reasons unclear, some stored red cell concentrates (RCC) have low hemolysis, while others have high hemolysis, which impacts quality consistency. To identify variables that influence hemolysis, routine quality control data for 42-days stored RCCs with corresponding donor information were analyzed.

Study Design and Methods: RCC and donor data were obtained from a national blood supplier. Regression models and analyses were performed on total cohort stratified by donor gender, and by high hemolysis ($\geq 90^{\text{th}}$ -percentile) versus control ($< 90^{\text{th}}$ -percentile) samples, including matching.

Results: Data included 1734 leukoreduced-RCCs (822 female; 912 male), processed by buffycoat-poor or whole blood filtration methods. Male RCCs had larger volume, hemoglobin content and higher hemolysis than female RCCs (median hemolysis, 0.24% versus 0.21%) (all $P < 0.0001$). Multivariable regression identified increased body mass index (BMI) and RCC variables were associated with higher hemolysis ($P < 0.0001$), along with older female age and buffycoat-poor processing method ($P < 0.002$). Logistic regression models comparing the high and control hemolysis subsets, matched for RBC component variables and processing method, identified overweight-obese BMI ($> 27 \text{ kg/m}^2$) in males remained the single donor-related variable associated with higher hemolysis ($P < 0.0001$); odds ratio, 3 (95% confidence interval [CI]: 1.3, 6.7), increasing to 4 (95%CI: 1.8, 8.6) for obese males (BMI $> 30 \text{ kg/m}^2$). Female donor obesity and older age trended towards higher hemolysis.

Conclusion: Donor BMI, gender and female age influence the level of hemolysis of 42-days stored RCCs. Other factors, not identified in this study, also influence the level of hemolysis.

Abstract word count: 240

Keywords: red cell concentrates, hemolysis, donor-related factors, body mass index, gender, quality

INTRODUCTION

Storage of red cell concentrates (RCCs) is associated with numerous physico-biochemical changes to RBCs, referred to as the RBC storage lesion, which may affect the function and survival of RBCs when transfused.¹ The extent of the RBC storage lesion can vary significantly between RCCs stored for the same duration, with some storing better than others.^{2,3} The reasons are likely multifaceted, including inherent donor-related heterogeneity and technical factors associated with blood collection, processing, storage or handling.⁴ Inadvertent production failures explain only a very small amount of the variability owing to wide-ranging systematic implementation of regulatory control and standardization of procedures in blood processing centers over the past 20-30 years.^{5,6} The clinical consequences of variability in storage quality of RCCs remain unclear;⁷ however it could introduce a significant unknown confounder to analyses and conclusions reached by RBC transfusion studies, particularly RBC storage clinical studies,^{8,9} such as recently completed large 'age of blood' randomized controlled trials.¹⁰ A better understanding of causes of variability is therefore needed to achieve greater consistency of RCC quality.

The most visible and easily measured *in vitro* quality indicator of the RBC storage lesion is the level of hemolysis, which is the proportion of hemoglobin (Hb) released from damaged or senescent RBCs into the suspension fluid, relative to the total Hb content and hematocrit (HCT).^{11,12} Hemolysis distribution curves of 42-days stored, pre-storage leukoreduced RCCs reported in two large studies - one from Canada¹³ and one from the USA¹⁴ - showed a positively-skewed normal distribution with a tapering tail of higher values beyond the maximum hemolysis limits specified by the Council of Europe (CoE) and USA Food and Drug Administration (FDA) maximum, i.e. <0.8% and <1%, respectively.^{15,16}

Differences in RCC production, such as processing methods and RBC preservative solutions,¹⁷ influence hemolysis levels, but so too does total volume and total RBC/Hb content.¹³ Male donor RCCs tend to be larger volume with higher Hb content and HCT than those from female donors, potentially altering the milieu within the RCC storage bag. Anecdotally, many producers of blood components assumed that these physical differences between male and female RCCs explained the tendency of higher storage-related hemolysis in male RCCs. However, recent studies, including from the large NHLBI REDS-III RBC-Omics project, have reported that donor gender independently influences the level of hemolysis of stored RCCs, together with other donor characteristics, such as donor age, ethnicity, sex hormone therapies, and inherited RBC traits.^{13,14,18-26}

To gain further insight into variables that influence RCC storability, we previously conducted a pilot analysis of routine QC hemolysis data of a national blood supplier, together with corresponding donor demographic, and identified increased donor body mass index (BMI) as a potentially significant factor associated with raised hemolysis levels.^{27,28} To our knowledge, donor BMI had not been reported as a contributing donor variable to stored RCC quality. We hypothesized that hemolysis in stored RCCs is influenced by donor BMI, together with donor gender and RCC variables. Here we present findings of our further analyses, including a conditional-matched sub-group analysis to control for RCC variables and thus identify key donor-related factors associated with RCC storage hemolysis.

MATERIAL AND METHODS

Study data

This was a retrospective study of pre-storage leukoreduced, non-irradiated RCCs, produced by Australia's national blood supplier between June 2008 and August 2010, that had been tested for hemolysis following 42 days of storage as part of the routine QC program. The study time-frame represented a period of minimal change in all aspects of the blood supplier's collection and processing operations at a national level. RCCs for the QC program were randomly selected on a monthly-testing schedule from the RCC inventory available for transfusion. The study was approved by the blood supplier's Human Research Ethics Committee.

Donor information, RCC and hemolysis data were extracted from the blood supplier's national donor and QC databases in May 2013. Only RCCs that met national QC criteria, excluding the hemolysis limit, were included (Table 1). RCCs were produced at nine of the blood supplier's laboratories around Australia. Donor body mass index (BMI) was calculated from donor height and weight records according to the standard equation.²⁹

RCC processing

All RCC were produced from whole blood (WB) donations ($450 \text{ mL} \pm 10\%$) held at 20 to 24°C and processed within 24 h. During the study period, two WB collection systems were used: 1) 'top-and-bottom processing' with an in-line RCC leukoreduction filter (Composelect T3922; Fresenius-Kabi, Bad Homburg, Germany), herein called the buffycoat-poor red cell (BCRC)-filter method, and 2) WB-leukoreduction filter system (Leukotrap WBF3; Pall Medical, Portsmouth, UK), herein called the WB-filter method. Both collection systems contained citrate-phosphate-dextrose anti-coagulant (63 mL)

and saline-adenine-glucose-mannitol (SAGM) additive solution (100 mL). All WB donations were centrifuged at 5,005 xg for 10 min at 22°C followed by semi-automated component separation (Optipress II; Baxter, Maurepas, France). RCCs were stored at 2 to 6°C and sampled on day 42 of storage. Total Hb and HCT were measured by automated hematology analyzer (Cell Dyn 3200, Abbott, Santa Clara, CA). Supernatant for Hb testing was prepared by centrifugation at 1,400 xg for 20 minutes and stored frozen. Supernatant Hb was measured by the blood supplier's QC spectrophotometric method,³⁰ and hemolysis (%) calculated according to the standard equation.¹²

Statistical analysis

All statistical analyses were performed using Stata software (version 14, Stata Corporation, College Station, TX). Continuous data were summarized as number (n), mean, SD, median, minimum-maximum. Categorical data were summarized using frequency tabulations (n (%)). Although a stratified sampling design was used with RCCs being obtained from nine processing sites, the design effect was negligible ($deff \approx 1.00$), therefore data from all sites were pooled. Mann-Whitney or Kruskal-Wallis rank tests were used to determine differences between groups for continuous variables and chi-squared and/or Fisher's Exact test for categorical variables. Significance was defined as $P < 0.05$.

Multivariable linear regression was used to model the level of hemolysis. Hemolysis values were transformed to natural logarithm (ln) to satisfy regression normality and homogeneity assumptions. Separate regression models were fitted for the male and female cohorts. Donor characteristics (age, BMI, Hb, ABO blood group), RCC characteristics (volume, HCT) and processing method were included

as explanatory variables. The variable, *Total Hb of RBC component*, was highly correlated with the volume variable, therefore was not included in any regression models.

To model the odds that a RCC would have high hemolysis levels, defined as RCCs in the $\geq 90^{\text{th}}$ -percentile of the hemolysis distribution for each gender, the odds were compared to the odds of that RCC being in the control group ($< 90^{\text{th}}$ -percentile of the hemolysis distribution). Categorized variables (based on quartiles in the control group) for the characteristics *Donor age*, *Donor BMI*, *Donor Hb*, *Volume of RBC component* and *HCT of RBC component* and nominal variables, *Donor ABO blood group* and *Processing method*, were used as explanatory variables in the logistic regression models. The odds ratio (OR) and test-based 95% confidence intervals (95% CI) were used to characterize the association between explanatory variables and the odds of hemolysis. Variables were first entered individually (unadjusted OR) and then were all included in a **multivariable** model to determine the multifactorially adjusted odds ratio (MVOR). To evaluate presence of a monotonic linear trend, categorical variables were fitted as an ordinal variable. The P-value for evaluating whether the resulting slope coefficient differed statistically from zero (referred to as 'P-trend' in the relevant Tables) was reported.

To separate the effects of donor characteristics from RCC variables and processing methods, the high hemolysis samples were matched with up to five control samples processed using the same method (BCRC-filter or WB-filter) and with similar values for *Volume of RBC component*, *HCT of RBC component* and *Total Hb of RBC component* using a Mahalanobis distance measure (Mahapick module, Stata software). **The Mahalanobis distance measures similarity in the matching variables between two samples, where 0 indicated two samples with exactly the same values for the matching**

variables, ≤ 1 indicated very close matches, and large values indicated extreme mismatch between samples. Selection of matches was done independently for each high hemolysis sample; consequently, a given control sample may have been matched to more than one high hemolysis sample. Conditional logistic regression models were then used to test the effects of the donor characteristics (*Donor age, Donor BMI, Donor Hb, Donor ABO blood group*).

RESULTS

For the study period, complete records for 1995 RCCs were available. Of these, 238 (11.9%) did not meet all study inclusion criteria listed in Table 1, and were excluded. The included dataset consisted of 1757 RCCs from 1734 donors (822 female; 912 male). Twenty-two donors had hemolysis data available for more than one donation; 21 donors with two donations and one donor with three donations. Comparison of intra-donor hemolysis variation showed good consistency overall, although RCCs from the two donors with highest hemolysis levels, donors #21 and #22, had wider differences (see **Supplementary Figure S1**). The intra-class correlation coefficient between donations was 0.82 when data for donors #21 and #22 were excluded. Only the first donation from these repeat donors was used in subsequent analyses (n=1734).

Compared to the national blood donor panel, the study cohort had a higher representation of male donors (47.6% versus 52.4%, respectively; $P < 0.0001$). This was due in part to a larger number of female RCCs that did not meet the national QC acceptance limits compared to male RCCs. There were higher proportions of blood group B and AB in the study cohort, which was expected due to lower issue rates of these blood types and therefore higher probability of availability for QC testing of expired RCCs ($P < 0.0001$) (**Supplementary Figure S2**).

Table 2 summarizes the donor cohort characteristics. Distribution of ABO blood groups was similar among male and female donors ($P = 0.5$). Male donors had higher median BMI compared to female donors (BMI 27.3 kg/m² versus 26.1 kg/m², respectively; $P < 0.0001$). By standard BMI classifications,²⁹ 47.5% of male donors and 34.9% of female donors were overweight (BMI 25 to 29.9

kg/m²) (P <0.0001) and approximately 28% of both male and female donors were obese (BMI ≥30 kg/m²) (P = 0.75) (**Supplementary Table S1**). For all other donor variables (**Table 2**), male donors had higher median values when compared to female donors.

For all RBC component characteristics (hemolysis, volume, total Hb and HCT), mean and median values were higher for male RCCs than female (P <0.0001) (**Table 2**). A significantly higher proportion of male RCCs were processed by the WB-filter method (P <0.0001) (**Table 2**). Female and male RCCs produced by the WB-filter method were significantly larger in volume, with higher total Hb and HCT compared to RCCs produced by the BCRC-filter method (P <0.0001) (**Table 3**).

Figure 1A shows the distribution of the hemolysis values. Fifty-one (2.9%) samples exceeded the 0.8% hemolysis CoE limit and 26 (1.5%) exceeded the 1.0% hemolysis FDA limit. A higher proportion of male RCCs exceeded the 0.8% hemolysis CoE limit compared to female RCCs (37 (4.1%) versus 14 (1.7%), respectively; P =0.004). **Of the RCCs with >0.8% hemolysis, a higher proportion were from obese donors (BMI ≥30 kg/m²) compared to RCCs with acceptable hemolysis levels (≤0.8% hemolysis); this difference was statistically significant for male RCCs (P =0.01) (**Figure 1B**). Overall, frequency of higher hemolysis levels in RCCs increased significantly with rising BMI of female and male donors (both P <0.0001) (**Figure 1C and 1D**, respectively).**

Modeling the level of hemolysis

To clarify the relationship between hemolysis level, donor gender, **BMI** and **other** possible explanatory variables, each continuous explanatory variable was split into four categories at its quartiles, by gender. **Statistical-based stratification of variables, rather than pre-specified**

categories, was used for cohort-balanced analysis. Mean hemolysis values were calculated for each category and plotted against the quartiles (**Figure 2**) (tabulated data are available in **Supplementary Table S2**). Note that for both genders, the 4th quartile (Q4) for the BMI variable approximated the standard definition of obesity ($\text{BMI} \geq 30 \text{ kg/m}^2$).²⁹ As there were significant gender differences for hemolysis and many of the other explanatory variables in the regression analysis, separate multiple regression models were fitted for the male and female cohorts. The variable, *Duration since previous donation*, was not included in the models as no significant differences in hemolysis levels were found for RCCs from donations with shorter duration than median duration versus those with longer duration than the median, nor the not available group (10.4%), for male and female cohorts (**Supplementary Table S3**).

For the female cohort, older ages, increased BMI and lower donor Hb levels were donor characteristics associated with higher hemolysis levels (**Table 4**). Of the RCC variables, larger volume, higher HCT and processing by the BCRC-filter method were associated with higher hemolysis levels (**Table 4**). Female RCC processed by the BCRC-filter method were estimated to have hemolysis levels 1.64-times higher than the WB-filter method (provided all other explanatory variables remained constant). For the male cohort, increased BMI was the only donor characteristic positively associated with hemolysis ($P < 0.0001$) (**Table 4**). Of the RCC variables, larger volume and processing by the BCRC-filter method were positively associated with higher hemolysis levels, whilst higher HCT values were associated with lower hemolysis levels (**Table 4**). Male RCC processed by the BCRC-filter method were estimated to have hemolysis levels 1.26-times higher than the WB-filter method (provided all other explanatory variables remain constant). However, considerable unexplained variation in hemolysis levels remained as the female and male regression models

accounted for only 17.2% and 8.6% of the variation, respectively (**Table 4** and **Supplementary Figures S3 and S4**).

Modeling the risk of hemolysis

High hemolysis samples were defined as those RCCs in the $\geq 90^{\text{th}}$ -percentile of the hemolysis distribution for that gender. For female RCCs, this ranged from 0.47% to 1.72% (n=82) and for male RCCs, it ranged from 0.55% to 2.38% (n=91). **Table 5** presents the prevalence of high hemolysis by donor characteristics, RCC variables for female and male cohorts along with the MVOR estimates (unadjusted ORs are available in **Supplementary Table S4**).

For females, **multivariable** logistic regression analysis identified that risk of hemolysis in RCCs increased with increasing donor age (P-trend = 0.014), higher donor BMI (P-trend = 0.0006), increasing RCC volumes (P-trend <0.0001) or processed by BCRC-filter method (OR = 2.3, 95% CI = (1.1, 4.5), P = 0.021). Risk of hemolysis in RCCs decreased as donor Hb increased or HCT levels >0.58 L/L (OR = 0.5) (**Table 5**). For males, risk of hemolysis in RCCs increased as donor BMI increased (P-trend = 0.0008), RCC volumes increased (P-trend <0.0001) or processed by BCRC-filter method (OR = 2.9, 95% CI = (1.4, 5.8), P = 0.0029). Risk of hemolysis decreased with increasing HCT of the RCC (P-trend = 0.0018) (**Table 5**). **Comparison of the proportion of RCCs in the high hemolysis and control groups, according to standard BMI classification,²⁹ is available in Supplementary Figure S5.** For the total study cohort, 43% of RCCs in the high hemolysis groups were from obese donors (BMI ≥ 30 kg/m²) compared to 26% in the control groups (P <0.0001).

Matched samples sub-group analysis

To separate the effect of donor characteristics from RCC and processing variables, high hemolysis RCCs (as defined above) were matched with up to five control RCCs processed by the same method and with similar RCC volume, HCT and total Hb. For female high hemolysis RCCs, 79 were matched with five controls and the remaining three were matched with only one control each. For male high hemolysis RCCs, 86 were matched with five controls, one with four controls, two with three controls, one with two controls and no controls could be matched for one high hemolysis RCC. **Ninety percent of the samples used as matched control samples had Mahalanobis distances less than 0.5, and the maximum score used for matching was 1.6. This represented excellent matching of control samples to high hemolysis samples, as evidenced by** distribution plots of matching variables **(Supplementary Figures S6 and S7 for female and male RCCs, respectively).**

Table 6 shows frequency distributions of high hemolysis RCCs and their matched controls, and MVOR estimates by donor characteristics for females and males. Unadjusted ORs are available in **Supplementary Table S5**. For females, **multivariable** conditional logistic regression analysis identified that risk of hemolysis in RCCs increased with increasing donor age (P-trend = 0.068) or with higher donor BMI (P-trend = 0.031); and decreased with increasing donor Hb at time of donation. For males, only higher donor BMI was associated with an increased risk of hemolysis (P-trend <0.0001). RCCs from male donors whose BMI ranged between 27.3 to 30.6 kg/m² had three-times the risk of hemolysis. The risk increased to four-times when male donor BMI was >30.7 kg/m² (**Table 6**).

DISCUSSION

Using routine QC hemolysis data together with manufacturing and donor demographic information, donor BMI in overweight and obese ranges were identified to be associated with high hemolysis in RCCs at 42 days of storage, particularly for male donors. Logistic regression modelling showed that RCCs from male donors with BMI $>27 \text{ kg/m}^2$ (i.e. mid-range of the overweight BMI classification) had approximately three-times MVOR of having hemolysis levels $\geq 90^{\text{th}}$ -percentile of the hemolysis distribution curve; the MVOR strengthened in the conditional logistic regression model. Similar results were found for RCCs from female donors; however, compared to male donors, MVORs were lower and weakened in the conditional logistic regression model. Overall, 43% of RCCs in the high hemolysis group were from obese donors (BMI $\geq 30 \text{ kg/m}^2$), compared to 26% in the control group ($P < 0.0001$). There was a signal for an association of higher hemolysis in RCCs from older female donors compared to younger female donors, but did not retain significance in the conditional logistic regression model. To our knowledge this is the first report that has identified an association of donor BMI with hemolysis levels in stored RCC. Nevertheless, additional factors not specifically identified in this study also influence hemolysis in stored RCCs.

The prevalence of obesity among Australian adults (≥ 18 years) has steadily increased over the past several decades; in 1995, prevalence was reported as 18.9%, in 2007-2008 it was 24.3% and in latest data (2017-2018) has risen further to 30.8%.³¹ Although blood donors represent a healthy adult population and must pass mandatory health screening prior to donation, the blood donor population is inevitably heterogeneous. For donor safety, a minimum body weight is specified, but typically no maximum weight limit.⁴ BMI *per se* is not a standard criterion for blood donor eligibility.

Our findings indicated that not all RCC from obese donors had higher hemolysis. A possible explanation, at least in part, may be found in the concept of metabolic health phenotypes and the relationship with predisposition for healthy or unhealthy body fat distribution in non-obese and obese adults.^{32,33} Storage of body fat centrally within the abdominal cavity or around and within internal organs, rather than subcutaneously, is characteristic of the metabolically-unhealthy phenotype, and can be exhibited in non-obese and obese individuals. Conversely, an estimated one-third of obese individuals appear metabolically healthy, although they have a heightened risk of becoming metabolically unhealthy.³⁴ The metabolically unhealthy phenotype has an increased risk of cardiovascular disease, Type-2 diabetes and other serious conditions, all of which are associated with potentially adverse immunological, inflammatory and vascular changes. Such changes are known to affect RBC properties.³⁵⁻³⁹ Detailed metabolic signatures that differentiate healthy and unhealthy changes in cellular metabolic pathways of obese individuals are being identified using metabolomics technologies.⁴⁰ Similar approaches could be applied to identify metabolic signatures in blood donors that correlate with RCC storage quality and transfusion efficacy.

Regarding the influence of other donor characteristics and RCC variables, our findings have similarities and differences to those reported by recent large studies, notably by Canadian Blood Services (CBS) and the RBC-Omics project.^{13,14} Male gender consistently features as a significant donor characteristic associated with higher hemolysis levels in stored RCCs. However, comparison between studies to assess the influence of RCC variables is hampered by differences in processing procedures, blood collection/storage systems, as well as statistical methodologies for unadjusted and adjusted analyses.^{13,41,42} In the RBC-Omics study, RCCs were produced by WB-filter methods and stored in AS-1 or AS-3 solutions, different to the SAGM-RCCs in this study.⁴²⁻⁴⁴ While CBS produces

SAGM-RCCs by the BCRC-filter and WB-filter methods, there are many differences in the specific protocols compared to those used in Australia. These include different manufacturers of the blood collection/storage systems and leukoreduction filters, WB holding conditions and centrifugation settings.^{13,41} Compared to CBS, the Australian protocol has higher *g*-force centrifugation, which possibly results in less residual plasma within the packed RBCs. Furthermore, the Australian protocol uses the same centrifugation conditions for all RCCs, whereas CBS uses separate centrifugation settings for RCCs processed by the BCRC-filter and WB-filter methods. Our multi-regression modeling indicated that the BCRC-filtered processing method is associated with higher hemolysis levels; however, to our knowledge adjusted analyses have not been reported for the Canadian data.

The clinical implications of transfusion of RCCs with high hemolysis are not well understood. Transfusion of increased amounts of cell-free Hb, including Hb encapsulated in RBC-derived microparticles, is capable of modulating the immune and vascular responses, which could have adverse consequences in certain clinical scenarios.⁴⁵⁻⁴⁹ Nevertheless, association of higher donor BMI and increased levels of hemolysis in stored RCCs raises questions about the resilience and transfusion efficacy of intact RBCs in these components, particularly if adverse changes persist in RBC metabolic profiles.

A strength of our study was that the dataset came from a national blood **supplier** that has fully standardized its operating procedures and is one of few blood **suppliers** that routinely tests a portion of its RCC inventory for end-of-storage hemolysis. Intra-donor reproducibility of hemolysis levels was very good in the sub-set of donors with results available for two RCCs during the study period. Other studies have also reported relatively high intra-donor consistency of RCC hemolysis for two

temporally separate blood donations from the same individual,^{50,51} which supports the notion of inherent donor factors. However, the RBC-Omics recalled blood donor sub-study reported poor intra-donor consistency of storage-related hemolysis levels in RCCs.⁵²

Our study has limitations. The sample size of the female donor cohort may have been insufficient to elucidate clear associations of increased BMI and older age with higher hemolysis. Many health and lifestyle-related donor variables that potentially influence resilience of RBCs to stresses induced by blood processing and storage are not recorded or are unknown. For example, diet choices, level of physical fitness, excesses in alcohol consumption, smoking, sex hormone therapies, are all known to influence RBC parameters,^{22,23,53-62} particularly via altered oxidative stress levels. BMI is an imprecise measure of healthy versus unhealthy body mass; more accurate measures that include waist circumference,⁶³ were not recorded. Donor ethnicity/race was not recorded; ethnic genetic background has been associated with susceptibility to hemolysis during RCC storage in the RBC-Omics study.¹⁴ However, Australia's blood donor panel is predominantly of Anglo-Celtic and European decent, with very small proportions of Asian and other ethnicities; thus ethnic variation is unlikely to have influenced our findings. Frequency of donation records were not collected, other than the duration since the previous WB donation relative to the subject donation. Indeed, findings from the RBC-Omics study indicated no significant effect of donation frequency on the level of storage-related hemolysis,⁶⁴ despite a shorter permitted inter-donation frequency of 56 days, compared to 84 days in Australia.⁴ The time-period of our study (2008-2010) may not reflect the current demographic profile of the Australian blood donor panel, particularly prevalence of obesity; however obesity-related pathology and that of any other underlying health conditions are unlikely to have significantly changed, thus nullifying a perceived limitation.

In conclusion, our study identified donor BMI as a significant donor variable associated with the level of hemolysis in stored RCCs. Further studies are needed to confirm and extend these results in order to obtain a comprehensive understanding of donor-related factors that influence stored RCC quality. More detailed donor health and lifestyle information would be beneficial for future studies.

ACKNOWLEDGEMENTS

We thank staff of the Process Control laboratories for sharing their hemolysis data, particularly Simone Enticott, Clare Hughes and Anthea Cheng; and Perfecto Diaz for extracting the national donor database information.

REFERENCES

1. Yoshida T, Prudent M, D'Alessandro A. Red blood cell storage lesion: causes and potential clinical consequences. *Blood Transfus* 2019;17:27-52.
2. Hess JR. Scientific problems in the regulation of red blood cell products. *Transfusion* 2012;52:1827-35.
3. D'Alessandro A, Zimring JC, Busch M. Chronological storage age and metabolic age of stored red blood cells: are they the same?. *Transfusion* 2019;59:1620-23.
4. Sparrow RL. Red blood cell components: time to revisit the sources of variability. *Blood Transfus* 2017;15:116-25.
5. Kim DU. The quest for quality blood banking program in the new millennium the American way. *Int J Hematol* 2002;76(Suppl 2):258-62.
6. Slopecki A, Smith K, Moore S. The value of Good Manufacturing Practice to a Blood Service in managing the delivery of quality. *Vox Sang* 2007;92:187-96.
7. Ning S, Heddle NM, Acker JP. Exploring donor and product factors and their impact on red cell post-transfusion outcomes. *Transfus Med Rev* 2018;32:28-35.
8. Middelburg RA, van de watering LMG, van der Bom JG. Blood transfusions: good or bad? Confounding by indication, an underestimated problem in clinical transfusion research. *Transfusion* 2010;50:1181-3.
9. Pereira A. Will clinical studies elucidate the connection between the length of storage of transfused red blood cells and clinical outcomes? An analysis based on the simulation of randomized controlled trials. *Transfusion* 2013;53:34-40.
10. McQuilten ZK, French CJ, Nichol A, et al. Effect of age of red cells for transfusion on patient outcomes: a systematic review and meta-analysis. *Transfus Med Rev* 2018;32:77-88. [published correction, *Transfus Med Rev* 2020;34:138-9].
11. Hess JR (for the BEST Collaborative). Measures of stored red blood cell quality. *Vox Sang* 2014;107:1-19.
12. Sowemimo-Coker SO. Red blood cell hemolysis during processing. *Transfu Med Rev* 2002;16:46-60.
13. Jordan A, Chen D, Yi QL, et al. Assessing the influence of component processing and donor characteristics on quality of red cell concentrates using quality control data. *Vox Sang* 2016;111:8-15.
14. Kanas T, Lanteri MC, Page GP, et al. Ethnicity, sex, and age are determinants of red blood cell storage and stress hemolysis: results of the REDS-III RBC-Omics study. *Blood Adv* 2017;1:1132-41.
15. AABB Standard Program Committee. Standards for blood banks and transfusion services. 32nd ed. Bethesda: AABB; 2020.

16. European Directorate for the Quality of Medicines & HealthCare. Guide to the preparation, use and quality assurance of blood components: recommendation. 20th ed: Strasborg: Council of Europe Publishing; 2020
17. Hess JR, Sparrow RL, van der Meer PF, et al. Red blood cell hemolysis during blood bank storage: using national quality management data to answer basic scientific questions. *Transfusion* 2009;49:2599-2603.
18. Raval JS, Waters JH, Seltsam A, et al. The use of the mechanical fragility test in evaluating sublethal RBC injury during storage. *Vox Sang* 2010;99:325-31.
19. Raval JS, Waters JH, Seltsam A, et al. Menopausal status affects the susceptibility of stored RBCs in mechanical stress. *Vox Sang* 2011;100:418-21.
20. Francis RO, Jhang JS, Pham HP, et al. Glucose-6-phosphate dehydrogenase deficiency in transfusion medicine: the unknown risks. *Vox Sang* 2013;105:271-82.
21. van 't Erve TJ, Wagner BA, Martin SM, et al. The heritability of hemolysis in stored human red blood cells. *Transfusion* 2015;55:1178-85.
22. Kanas T, Sinchar D, Osei-Hwedie D, et al. Testosterone-dependent sex differences in red blood cell hemolysis in storage, stress, and disease. *Transfusion* 2016;56:2571-83.
23. Fang F, Hazegh K, Sinchar D, et al. Sex hormone intake in female blood donors: impact on haemolysis during cold storage and regulation of erythrocyte calcium influx by progesterone. *Blood Transfus* 2019;17:263-73.
24. van 't Erve TJ, Wagner BA, Martin SM, et al. The heritability of metabolite concentrations in stored human red blood cells. *Transfusion* 2014;54:2055-63.
25. van 't Erve TJ, Doskey CM, Wagner BA, et al. Heritability of glutathione and related metabolites in stored red blood cells. *Free Radic Biol Med* 2014;76:107-13.
26. Weisenhorn EM, van 't Erve TJ, Riley NM, et al. Multi-omics evidence for inheritance of energy pathways in red blood cells. *Mol Cell Proteomics* 2016;15:3614-23.
27. Sparrow RL, Payne KA. Donor demographics of red blood cell units with very low compared to very high hemolysis at 42-days of storage are significantly different. *Transfusion* 2014;54(Suppl't 2):41A.
28. Sparrow RL, Payne KA. Donor factors, rather than physical variables of red blood cell components, determine the level of hemolysis at 42-days of storage. *Transfusion* 2015;55(Suppl't 3):55A.
29. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. WHO technical report series 894. 2000 Geneva:WHO. http://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/ Accessed October 4, 2020.
30. Blakney G, Dinwoodie A. A spectrophotometric scanning technique for the rapid determination of plasma hemoglobin. *Clin Biochem* 1975;8:96-102.
31. Australian Institute of Health and Welfare (AIHW). Overweight and obesity: an interactive insight. Time trends. AIHW Web report, updated July 19, 2019. Australian Government, AIHW,

Canberra, ACT, Australia. <https://www.aihw.gov.au/reports/overweight-obesity/overweight-and-obesity-an-interactive-insight/contents/time-trends>. Accessed October 4, 2020.

32. Jung CH, Lee WJ, Song KH. Metabolically healthy obesity: a friend or foe?. *Korean J Intern Med* 2017;32:611-21.
33. Stefan N. Causes, consequences, and treatment of metabolically unhealthy fat distribution. *Lancet Diabetes Endocrinol* 2020;8:616-27.
34. Bell JA, Hamer M. Healthy obesity as an intermediate state of risk: a critical review. *Expert Rev Endocrinol Metab* 2016;11:403-13.
35. Brun JF, Varlet-Marie E, Fedou C, Raynaud de Mauverger E. Are metabolically healthy obese patients also hemorheologically healthy? *Clin Hemorheol Microcirc* 2015;61:39-46.
36. Zeng NF, Mancuso JE, Zivkovic AM, et al. Red blood cells from individuals with abdominal obesity or metabolic abnormalities exhibit less deformability upon entering a constriction. *PLoS One* 2016;11:e0156070.
37. Namazi G, Asa P, Sarrafzadegan N, Pourfarzam M. Decreased Na⁺/K⁺-ATPase activity and altered susceptibility to peroxidation and lipid composition in the erythrocytes of metabolic syndrome patients with coronary artery disease. *Ann Nutr Metab* 2019;74:140-8.
38. Farah R, Khamisy-Farah R. Significance of MPV, RDW with the presence and severity of metabolic syndrome. *Exp Clin Endocrinol Diabetes* 2015;123:567-70.
39. Buys AV, Van Rooy MJ, Soma P, et al. Changes in red blood cell membrane structure in type 2 diabetes: a scanning electron and atomic force microscopy study. *Cardiovasc Diabetol* 2013;12:25.
40. Rangel-Huerta OD, Pastor-Villaescusa B, Gil A. Are we close to defining a metabolomic signature of human obesity? A systematic review of metabolomics studies. *Metabolomics* 2019;15:93.
41. Shih AW, Apelseh TO, Cardigan R, et al. Not all red cell concentrate units are equivalent: international survey of processing and in vitro quality data. *Vox Sang* 2019;114:783-94.
42. D'Alessandro A, Culp-Hill R, Reisz JA, et al. Heterogeneity of blood processing and storage additives in different centers impacts stored red blood cell metabolism as much as storage time: lessons from REDS-III-Omics. *Transfusion* 2019;59:89-100.
43. Sparrow RL, Sran A, Healey G, et al. In vitro measures of membrane changes reveal differences between red blood cells stored in saline-adenine-glucose-mannitol and AS-1 additive solutions: a paired study. *Transfusion* 2014;54:560-8.
44. Rolfsson Ó, Sigurjonsson ÓE, Magnúsdóttir M, et al. Metabolomics comparison of red cells stored in four additive solutions reveals differences in citrate anticoagulant permeability and metabolism. *Vox Sang* 2017;112:326-335.
45. Rifkind JM, Mohanty JG, Nagababu E. The pathophysiology of extracellular hemoglobin associated with enhanced oxidative reactions. *Front Physiol.* 2015;5:500.
46. Rapido F. The potential adverse effects of haemolysis. *Transfus Apher Sci* 2017;15:218-21.

47. Donadee C, Raat NJ, Kanias T, et al. Nitric oxide scavenging by red blood cell microparticles and cell-free hemoglobin as a mechanism for the red cell storage lesion. *Circulation* 2011;124:465-76.
48. Almizraq RJ, Seghatchian J, Acker JP. Extracellular vesicles in transfusion-related immunomodulation and the role of blood component manufacturing. *Transfus Apher Sci* 2016;55:281-91.
49. Bouchard BA, Orfeo T, Keith HN, et al. Microparticles formed during storage of red blood cell units support thrombin generation. *J Trauma Acute Care Surg* 2018;84:598-605.
50. McAteer MJ, Dumont LJ, Cancelas J, et al. Multi-institutional randomised control study of haemolysis in stored red cell units prepared manually or by an automated system. *Vox Sang* 2010;99:34-43.
51. Tarasev M, Alfano K, Chakraborty S, Light L, Doeden K, Gorlin JB. Similar donors—similar blood? *Transfusion* 2014;54:933-41.
52. Lanteri MC, Kanias T, Keating S, et al. Intradonor reproducibility and changes in hemolytic variables during red blood cell storage: results of recall phase of the REDS-III RBC-Omics study. *Transfusion* 2019;59:79-88.
53. Unruh D, Srinivasan R, Benson T, et al. Red blood cell dysfunction induced by high-fat diet: potential implications for obesity-related atherosclerosis. *Circulation* 2015;132:1898-908.
54. Tutino V, De Nunzio V, Caruso MG, et al. Aerobic physical activity and a low glycemic diet reduce the AA/EPA ratio in red blood cell membranes of patients with NAFLD. *Nutrients* 2018;10:1299.
55. Giardina S, Sala-Vila A, Hernández-Alonso P, et al. Carbohydrate quality and quantity affects the composition of the red blood cell fatty acid membrane in overweight and obese individuals. *Clin Nutr* 2018;37:481-7.
56. D'Alessandro A, Fu X, Reisz JA, et al. Stored RBC metabolism as a function of caffeine levels. *Transfusion* 2020;60:1197-211.
57. Bertolone L, Roy MK, Hay AM, et al. Impact of taurine on red blood cell metabolism and implications for blood storage. *Transfusion* 2020;60:1212-26.
58. Maturu P, Vaddi DR, Pannuru P, et al. Modification of erythrocyte membrane proteins, enzymes and transport mechanisms in chronic alcoholics: an in vivo and in vitro study. *Alcohol Alcohol* 2013;48:679-86.
59. Pretorius E, du Plooy JN, Soma P, et al. Smoking and fluidity of erythrocyte membranes: a high resolution scanning electron and atomic force microscopy investigation. *Nitric Oxide* 2013;35:42-6.
60. Stefanoni D, Fu X, Reisz JA, et al. Nicotine exposure increases markers of oxidant stress in stored red blood cells from healthy donor volunteers. *Transfusion* 2020;60:1160-74.
61. Pehlivanoglu B, Dikmenoglu N, Balkanci DZ. Effect of stress on erythrocyte deformability, influence of gender and menstrual cycle. *Clin Hemorheol Microcirc* 2007;37:301-8.

62. Chang S-H, Chang C-H, Yang M-C, et al. Effects of estrogen on glutathione and catalase levels in human erythrocyte during menstrual cycle. *Biomed Rep* 2015;3:266-8.
63. Venkatrao M, Nagarathna R, Patil SS, et al. A composite of BMI and waist circumference may be a better obesity metric in Indians with high risk for type 2 diabetes: An analysis of NMB-2017, a nationwide cross-sectional study. *Diabetes Res Clin Pract* 2020;161:108037.
64. Kanas T, Stone M, Page GP, et al. Frequent blood donations alter susceptibility of red blood cells to storage- and stress-induced hemolysis. *Transfusion* 2019;59:67-78.

Table 1. Study inclusion requirements: donor demographic and RBC component information

Variable	Requirement or limit*
<i>Donor-related</i>	
Gender, age, height, weight, ABO blood type	Required
Hb at donation (g/dL)	Male: 12.5 – 18.5 Female: 11.5 – 16.5
Duration since previous whole blood donation (days)†	≥ 84
<i>RBC component-related</i>	
Hemolysis, blood collection pack type, processing method, processing site, dates of blood collection and sample testing, spectrophotometric method used for plasma hemoglobin measurement	Required
Total Hemoglobin (g/unit)	≥ 40
Volume (mL)	> 220
Hematocrit (L/L)	0.5 – 0.7

* Limits are those that operated at the time of blood donation

† Absence of this information was not an exclusion criteria

Table 2. Descriptive statistics for donor and RBC component characteristics

Variable	Female Donors (n = 822)	Male Donors (n = 912)	Total Donors (n = 1734)	P-value
Donor characteristics				
Donor age				
Mean ± SD	44.4 ± 15.0	46.4 ± 14.5	45.4 ± 14.7	0.005 ^a
Median	47	48	47.5	
Minimum – Maximum	16 – 75	16 – 79	16 – 79	
Donor BMI				
Mean ± SD	27.4 ± 5.7	28.0 ± 4.5	27.7 ± 5.1	0.027 ^a
Median	26.1	27.3	26.9	
Minimum – Maximum	17.2 – 54.3	17.3 – 51.4	17.2 – 54.3	
Donor hemoglobin				
Mean ± SD	13.5 ± 0.9	15.0 ± 1.1	14.3 ± 1.2	< 0.0001 ^a
Median	13.5	15.0	14.2	
Minimum – Maximum	11.8 – 16.4	12.7 – 18.7	11.8 – 18.7	
Donor blood group				
A	230 (27.8%)	282 (30.8%)	512 (29.4%)	0.5 ^b
AB	130(16.1%)	143 (15.7%)	273 (15.9%)	
B	216 (25.9%)	215 (23.8%)	431 (24.8%)	
O	246 (30.0%)	272 (29.6%)	518 (29.8%)	
Duration since previous donation ^c				
Mean ± SD	261 ± 354	214 ± 276	236 ± 316	0.001 ^a
Median	113	104	109	
Minimum – Maximum	84 – 3566	84 – 2596	84 – 3566	
RBC component characteristics				
Hemolysis (%)				
Mean ± SD	0.26 ± 0.18	0.31 ± 0.24	0.29 ± 0.21	< 0.0001 ^a
Median	0.21	0.24	0.23	
Minimum – Maximum	0.02 – 1.72	0.02 – 2.38	0.02 – 2.38	
Volume of RBC component				
Mean ± SD	248 ± 16	275 ± 21	262 ± 23	< 0.0001 ^a
Median	245	272	257	
Minimum – Maximum	221 – 315	225 – 334	221 – 334	
Hematocrit of RBC component				
Mean ± SD	0.59 ± 0.03	0.63 ± 0.03	0.61 ± 0.04	< 0.0001 ^a
Median	0.59	0.63	0.61	
Minimum – Maximum	0.50 – 0.70	0.53 – 0.70	0.50 – 0.70	
Total Hemoglobin of RBC component				
Mean ± SD	46.1 ± 4.3	54.7 ± 6.0	50.6 ± 6.8	< 0.0001 ^a
Median	45.3	54.1	49.5	
Minimum – Maximum	40.0 – 63.2	40.9 – 71.8	40.0 – 71.8	

Variable	Female Donors (n = 822)	Male Donors (n = 912)	Total Donors (n = 1734)	P-value
Processing Method				
BCRC-filter (Fresenius)	707 (86.0%)	547 (60.0%)	1254 (72.3%)	< 0.0001 ²
WB-filter (Pall)	115 (14.0%)	365 (40.0%)	480 (27.7%)	

^a P-values are for the male donors versus female donors comparison and were determined using the Mann-Whitney Rank Sum test.

^b P-values are for the male donors versus female donors comparison and were determined using the Chi-squared test.

^c n= 1,553 for all donors (729 Female / 824 Male) since 181 donors were first time donors and so this variable was not defined.

Table 3. Comparison of RBC component variables produced by the buffycoat-red cell (BCRC) filter or whole blood (WB) filter methods

Variable	Donors and processing method					
	Female Donors (n=822) ^a			Male Donors (n=912)		
	BCRC-filter (Fresenius) (n=707)	WB-filter (Pall) (n=115)	P value ^b	BCRC-filter (Fresenius) (n=547)	WB-filter (Pall) (n=365)	P value ^b
Total Hemoglobin (g/unit)						
Mean ± SD	44.9 ± 3.0	53.3 ± 3.8	< 0.0001	51.2 ± 4.1	60.0 ± 4.1	< 0.0001
Median	44.6	53.1		51.2	59.9	
Minimum – Maximum	40.0 – 58.3	44.3 – 63.2		40.9 – 65.8	43.3 – 71.8	
Total Volume (mL)						
Mean ± SD	243 ± 10	278 ± 12	< 0.0001	262 ± 14	294 ± 13	< 0.0001
Median	243	277		261	295	
Minimum – Maximum	221 – 288	250 – 315		225 – 324	246 – 334	
Hematocrit (L/L)						
Mean ± SD	0.59 ± 0.02	0.62 ± 0.02	< 0.0001	0.62 ± 0.03	0.65 ± 0.02	< 0.0001
Median	0.58	0.62		0.62	0.65	
Minimum – Maximum	0.50 – 0.69	0.57 – 0.70		0.53 – 0.70	0.56 – 0.70	
Hemolysis (%)						
Mean ± SD	0.25 ± 0.19	0.27 ± 0.15	0.04	0.33 ± 0.26	0.29 ± 0.19	0.04
Median	0.2	0.23		0.25	0.24	
Minimum – Maximum	0.02 – 1.72	0.05 – 0.76		0.02 – 2.38	0.07 – 1.56	

^a Corresponding data points for male versus female were all p < 0.0001, except for percent hemolysis between male and female WB-filtered units (p = 0.5); determined by Mann-Whitney Rank Sum test.

^b P-values shown are BCRC-filter versus WB-filter processing methods for each gender, determined using the Mann-Whitney Rank Sum test.

Table 4. Multiple regression models for the level of hemolysis in a RBC component. The natural logarithm of hemolysis was used as the dependent variable.

Variable	Female Donors (n = 822)			Male Donors (n = 912)		
	Estimate	SE	P-value	Estimate	SE	P-value
Constant (%)	0.17 ^b	0.01	< 0.0001	0.23 ^c	0.02	< 0.0001
Donor characteristics						
Donor age (/10 years) ^a	1.04	0.01	0.0012	1.02	0.01	0.074
Donor BMI (/5 kg/m ²) ^a	1.09	0.02	< 0.0001	1.13	0.02	< 0.0001
Donor hemoglobin (/1 g/dL) ^a	0.93	0.02	0.0024	0.99	0.02	0.54
Donor blood group ^d						
AB	0.90	0.04	0.031	0.99	0.05	0.89
B	0.99	0.05	0.84	0.95	0.05	0.37
O	0.99	0.05	0.84	0.91	0.04	0.056
RBC component characteristics						
Volume of RBC component (/20 mL) ^a	1.56	0.08	< 0.0001	1.20	0.05	0.0001
Hematocrit of RBC component (/0.1 L/L) ^a	0.60	0.07	< 0.0001	0.63	0.06	0.0013
Processing method ^e						
BCRC-filter (Fresenius)	1.64	0.16	< 0.0001	1.26	0.10	0.0022
Adjusted R² statistic	0.17			0.086		

^a All continuous covariates were centred and rescaled prior to being included in the regression model.

^b The constant term is the estimated amount of hemolysis in a RBC sample from a 45-year old female donor, whose BMI was 27, hemoglobin at donation was 14.3 and blood group was A. The volume of the RBC sample was 260 mL and hematocrit was 0.6, and the donation was processed using the whole blood filter (Pall) method.

^c The constant term is the estimate amount of hemolysis in a RBC sample from a 45-year old male donor, whose BMI was 27, hemoglobin at donation was 14.3 and blood group was A. The volume of the RBC sample was 260 mL and hematocrit was 0.6, and was the donation was processed using the whole blood filter (Pall) method.

^d Reference category is blood group A.

^e Reference category for Processing method is WB-filter (Pall).

Table 5. Logistic regression modelling for the risk of hemolysis in a RBC component.*

FEMALES						MALES					
Quartile Data Range		High Hemolysis Samples (n = 82)	Control Samples (n = 740)	MVOR	P-value	Quartile Data Range		High Hemolysis Samples (n = 91)	Control Samples (n = 821)	MVOR	P-value
Donor characteristics											
Donor age											
Q1	16 – 30	14 (17.1%)	185 (25.0%)	1.0 (reference)		16 – 35	17 (18.7%)	211 (25.7%)	1.0 (reference)		
Q2	31 – 46	17 (20.7%)	188 (25.4%)	1.1 (0.5, 2.3)	0.84	36 – 47	23 (25.3%)	197 (24.0%)	1.2 (0.8, 1.7)	0.34	
Q3	47 – 56	25 (30.5%)	195 (26.4%)	1.6 (1.1, 2.3)	0.0097	48 – 57	26 (28.6%)	208 (25.3%)	1.3 (1.0, 1.8)	0.07	
Q4	57 – 75	26 (31.7%)	172 (23.2%)	1.7 (1.0, 2.8)	0.046	58 – 79	25 (27.5%)	205 (25.0%)	1.3 (0.7, 2.6)	0.43	
P-trend = 0.014						P-trend = 0.38					
Donor BMI											
Q1	17.2 – 23.1	12 (14.6%)	188 (25.4%)	1.0 (reference)		17.3 – 24.8	10 (11.0%)	201 (24.5%)	1.0 (reference)		
Q2	23.2 – 25.9	14 (17.1%)	181 (24.5%)	1.1 (0.5, 2.1)	0.87	24.9 – 27.0	15 (16.5%)	205 (25.0%)	1.4 (0.7, 2.8)	0.39	
Q3	26.0 – 30.1	24 (29.3%)	191 (25.8%)	1.7 (1.2, 2.5)	0.0058	27.1 – 30.2	28 (30.8%)	210 (25.6%)	2.4 (1.3, 4.5)	0.0040	
Q4	30.2 – 54.3	32 (39.0%)	180 (24.3%)	2.4 (1.5, 3.9)	0.0002	30.3 – 51.4	38 (41.8%)	205 (25.0%)	3.3 (1.5, 7.4)	0.0033	
P-trend = 0.0006						P-trend = 0.0008					
Donor hemoglobin											
Q1	11.8 – 12.8	21 (25.6%)	181 (24.5%)	1.0 (reference)		12.7 – 14.2	18 (19.8%)	214 (26.1%)	1.0 (reference)		
Q2	12.9 – 13.4	19 (23.2%)	184 (24.9%)	0.8 (0.3, 2.1)	0.63	14.3 – 14.9	18 (19.8%)	196 (23.9%)	0.9 (0.5, 1.8)	0.84	
Q3	13.5 – 14.1	26 (31.7%)	191 (25.8%)	0.8 (0.4, 1.6)	0.52	15.0 – 15.6	21 (23.1%)	209 (25.5%)	0.9 (0.5, 1.6)	0.74	
Q4	14.2 – 16.4	16 (19.5%)	184 (24.9%)	0.3 (0.1, 0.9)	0.023	15.7 – 18.7	34 (37.4%)	202 (24.6%)	1.2 (0.7, 1.9)	0.56	
P-trend = 0.013						P-trend = 0.69					
Donor blood group											

FEMALES						MALES					
	Quartile Data Range	High Hemolysis Samples (n = 82)	Control Samples (n = 740)	MVOR	P-value		Quartile Data Range	High Hemolysis Samples (n = 91)	Control Samples (n = 821)	MVOR	P-value
A		18 (22.0%)	212 (28.7%)	1.0 (reference)		A		24 (26.4%)	258 (31.4%)	1.0 (reference)	
AB		13 (15.9%)	117 (15.8%)	1.3 (0.7, 2.4)	0.50	AB		15 (16.5%)	128 (15.6%)	1.2 (0.7, 2.3)	0.48
B		21 (25.6%)	195 (26.4%)	1.1 (0.5, 2.6)	0.75	B		22 (24.2%)	193 (23.5%)	1.3 (0.7, 2.6)	0.39
O		30 (36.6%)	216 (29.2%)	1.6 (0.9, 2.8)	0.13	O		30 (33.0%)	242 (29.5%)	1.2 (0.5, 2.9)	0.61
					P-overall = 0.49						P-overall = 0.77

FEMALES						MALES					
Quartile Data Range		High Hemolysis Samples (n = 82)	Control Samples (n = 740)	MVOR	P-value	Quartile Data Range		High Hemolysis Samples (n = 91)	Control Samples (n = 821)	MVOR	P-value
RBC component characteristics											
Volume of RBC component											
Q1	221 – 236	7 (8.5%)	187 (25.3%)	1.0 (reference)		225 – 258	12 (13.2%)	210 (25.6%)	1.0 (reference)		
Q2	237 – 244	15 (18.3%)	191 (25.8%)	2.8 (0.7, 12.0)	0.17	259 – 271	19 (20.9%)	210 (25.6%)	2.0 (1.0, 3.8)	0.04	
Q3	245 – 254	24 (29.3%)	183 (24.7%)	5.9 (2.5, 13.5)	< 0.0001	272 – 292	38 (41.8%)	209 (25.5%)	8.5 (4.8, 15.2)	< 0.0001	
Q4	255 – 315	36 (43.9%)	179 (24.2%)	18.0 (6.2, 52.3)	< 0.0001	293 – 334	22 (24.2%)	192 (23.4%)	10.1 (3.5, 28.8)	< 0.0001	
				P-trend < 0.0001						P-trend < 0.0001	
Hematocrit of RBC Component											
Q1	0.50 – 0.57	25 (30.5%)	233 (31.5%)	1.0 (reference)		0.53 – 0.61	31 (34.1%)	261 (31.8%)	1.0 (reference)		
Q2	0.58 – 0.59	19 (23.2%)	214 (28.9%)	0.5 (0.3, 0.6)	< 0.0001	0.62 – 0.63	23 (25.3%)	199 (24.2%)	0.6 (0.2, 1.4)	0.21	
Q3	0.60 – 0.61	20 (24.4%)	166 (22.4%)	0.5 (0.3, 0.8)	0.0088	0.64 – 0.65	18 (19.8%)	184 (22.4%)	0.3 (0.2, 0.7)	0.0016	
Q4	0.62 – 0.70	18 (22.0%)	127 (17.2%)	0.5 (0.1, 1.8)	0.29	0.66 – 0.70	19 (20.9%)	177 (21.6%)	0.3 (0.1, 0.8)	0.012	
				P-trend = 0.29						P-trend = 0.0018	
Processing method											
WB-filter		14 (17.1%)	101 (13.7%)	1.0 (reference)		WB-filter		31 (34.1%)	334 (40.7%)	1.0 (reference)	
BCRC-filter		68 (82.9%)	639 (86.4%)	2.3 (1.1, 4.5)	0.021	BCRC-filter		60 (65.9%)	487 (59.3%)	2.9 (1.4, 5.8)	0.0029

* RCCs were stratified by high hemolysis ($\geq 90^{\text{th}}$ -percentile) of the hemolysis distribution curve and control samples were those with $< 90^{\text{th}}$ -percentile hemolysis.

Table 6. Conditional logistic regression on matched samples to separate the effect of donor variables from RBC component and processing variables.*

Quartile	FEMALES					MALES				
	Quartile Data Range	High Hemolysis Samples (n = 82)	Matched Samples (n = 398)	MVOR	P-value	Quartile Data Range	High Hemolysis Samples (n = 90)	Matched Samples (n = 442)	MVOR	P-value
Donor Age										
Q1	16 – 28	13 (15.9%)	102 (25.6%)	1.0 (reference)		16 – 35	16 (23.7%)	110 (24.9%)	1.0 (reference)	
Q2	29 – 47	20 (24.4%)	101 (25.4%)	1.5 (0.7, 3.4)	0.32	36 – 48	26 (25.4%)	109 (24.7%)	1.2 (0.6, 2.4)	0.56
Q3	48 – 56	23 (28.1%)	102 (25.6%)	1.9 (0.8, 4.3)	0.16	49 – 56	20 (24.8%)	112 (25.3%)	0.8 (0.4, 1.6)	0.52
Q4	57 – 75	26 (31.7%)	93 (23.4%)	2.2 (0.9, 5.4)	0.072	57 – 79	28 (26.1%)	111 (25.1%)	1.3 (0.6, 2.7)	0.46
				P-trend = 0.068					P-trend = 0.73	
Donor BMI										
Q1	17.2 – 23.3	12 (14.6%)	101 (25.4%)	1.0 (reference)		18.2 – 24.8	10 (22.7%)	111 (25.1%)	1.0 (reference)	
Q2	23.4 – 26.0	15 (18.3%)	99 (24.9%)	1.1 (0.5, 2.5)	0.89	24.9 – 27.2	16 (23.7%)	110 (24.9%)	1.4 (0.5, 3.5)	0.51
Q3	26.1 – 30.5	27 (32.9%)	98 (24.6%)	1.9 (0.9, 4.3)	0.11	27.3 – 30.6	28 (26.1%)	111 (25.1%)	3.0 (1.3, 6.7)	0.0090
Q4	30.6 – 54.1	28 (34.2%)	100 (25.1%)	2.1 (0.9, 4.6)	0.072	30.7 – 50.9	36 (27.4%)	110 (24.9%)	4.0 (1.8, 8.6)	0.0005
				P-trend = 0.031					P-trend < 0.0001	
Donor Hemoglobin										
Q1	11.9 – 13.0	25 (30.5%)	104 (26.1%)	1.0 (reference)		12.8 – 14.3	23 (24.3%)	106 (24.0%)	1.0 (reference)	
Q2	13.1 – 13.6	23 (28.1%)	102 (25.6%)	1.0 (0.5, 2.0)	0.89	14.4 – 15.0	14 (22.9%)	108 (24.4%)	0.6 (0.2, 1.5)	0.29
Q3	13.7 – 14.4	22 (26.8%)	103 (25.9%)	0.8 (0.4, 1.7)	0.62	15.1 – 16.0	38 (29.3%)	118 (26.7%)	1.9 (0.9, 4.1)	0.10
Q4	14.5 – 16.4	12 (14.6%)	89 (22.4%)	0.6 (0.2, 1.3)	0.20	16.1 – 18.4	15 (23.5%)	110 (24.9%)	0.7 (0.3, 1.7)	0.42
				P-trend = 0.16					P-trend = 0.99	
Donor Blood Group										

Quartile	FEMALES					MALES				
	Quartile Data Range	High Hemolysis Samples (n = 82)	Matched Samples (n = 398)	MVOR	P-value	Quartile Data Range	High Hemolysis Samples (n = 90)	Matched Samples (n = 442)	MVOR	P-value
A		18 (22.0%)	105 (26.4%)	1.0 (reference)			23 (24.1%)	105 (23.8%)	1.0 (reference)	
AB		13 (15.9%)	73 (18.3%)	1.0 (0.4, 2.4)	0.96		15 (16.9%)	75 (17.0%)	0.9 (0.4, 1.8)	0.69
B		21 (25.6%)	98 (24.6%)	1.3 (0.6, 2.6)	0.51		22 (22.4%)	97 (22.0%)	1.0 (0.5, 1.9)	0.94
O		30 (36.6%)	122 (30.7%)	1.6 (0.8, 3.0)	0.15		30 (36.7%)	165 (37.3%)	0.7 (0.4, 1.4)	0.34
					P-overall = 0.43					
										P-overall = 0.78

* RCC were stratified by high hemolysis ($\geq 90^{\text{th}}$ -percentile) of the hemolysis distribution curve and matched control samples were those with $< 90^{\text{th}}$ -percentile hemolysis, matched for RBC component volume, HCT and processing method.

Figure 1

Figure 2

FIGURE LEGENDS

Figure 1

Hemolysis distribution of the total study cohort comprised of 1734 42-days stored, pre-storage

leukocyte-reduced, non-irradiated SAGM-RCCs. (A) Frequency plot of the levels of hemolysis.

Median % hemolysis of the total cohort was 0.23% and mean was 0.29%. 51 (2.9%) RCCs exceeded the CoE maximum limit of 0.8% hemolysis (dashed line); 26 (1.5%) RCCs exceeded the FDA maximum limit of 1.0% hemolysis (dotted line). (B) Comparison of the proportion of RCCs with acceptable level

of hemolysis (<0.8%) versus RCCs with hemolysis that exceeded the CoE 0.8% maximum limit, by donor body mass index (BMI) category according to the World Health Organization classifications.²⁹

Data for RCCs from female donors (left-half of plot) and male donors (right-half of plot). BMI

categories: obese BMI ≥ 30 kg/m² (black), overweight BMI 25-29.9 kg/m² (dark grey), healthy BMI

18.5-24.9 kg/m² (light grey) and underweight BMI <18.5 kg/m² (white). Numbers shown within the

stack are the proportion (%) for each BMI category. The total number of RCCs for each subgroup is

indicated in the horizontal axis label. For male RCCs, there was a significantly higher proportion of

RCCs from obese donors that exceeded the maximum hemolysis limit compared to the acceptable

hemolysis group ($P = 0.01$, by chi-squared test). A similar trend was seen for female RCCs, however

was underpowered to reach statistical significance. (C) Dot distribution plots of % hemolysis of

female RCCs by donor BMI category, and (D) for male RCCs.. The sample size of each subgroup is

indicated in the horizontal axis label. The frequency of higher hemolysis levels progressively

increased with BMI, and the differences across BMI categories were statistically significant for RCCs

from female and male donors ($P < 0.0001$ for both, by Kruskal-Wallis test).

Figure 2

Mean levels of hemolysis by quartiles for the continuous donor characteristics and RCC-related variables, stratified by gender. Data on the quartile ranges and the hemolysis mean and standard deviation values are available in Supplementary Table S2. (A) donor age; (B) donor BMI; (C) donor haemoglobin level pre-donation; (D) volume of RBC component; (E) hematocrit of RBC component; (F) total haemoglobin content of RBC component.

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Sparrow, RL;Payne, KA;Adams, GG

Title:

Higher donor body mass index is associated with increased hemolysis of red blood cells at 42-days of storage: A retrospective analysis of routine quality control data

Date:

2020-11-24

Citation:

Sparrow, R. L., Payne, K. A. & Adams, G. G. (2020). Higher donor body mass index is associated with increased hemolysis of red blood cells at 42-days of storage: A retrospective analysis of routine quality control data. TRANSFUSION, 61 (2), pp.449-463. <https://doi.org/10.1111/trf.16203>.

Persistent Link:

<http://hdl.handle.net/11343/276644>