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Impact of blood manufacturing and donor characteristics on membrane water permeability and *in vitro* quality parameters during hypothermic storage of red blood cells

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ABSTRACT

Several factors have been proposed to influence the red blood cell storage lesion including storage duration, blood component manufacturing methodology, and donor characteristics [1,18]. The objectives of this study were to determine the impact of manufacturing method and donor characteristics on water permeability and membrane quality parameters.

Red blood cell units were obtained from volunteer blood donors and grouped according to the manufacturing method and donor characteristics of sex and age. Membrane water permeability and membrane quality parameters, including deformability, hemolysis, osmotic fragility, hematologic indices, supernatant potassium, and supernatant sodium, were determined on day 5 ± 2, day 21, and day 42. Regression analysis was applied to evaluate the contribution of storage duration, manufacturing method, and donor characteristics on storage lesion.

This study found that units processed using a whole blood filtration manufacturing method exhibited significantly higher membrane water permeability throughout storage compared to units manufactured using red cell filtration. Additionally, significant differences in hemolysis, supernatant potassium, and supernatant sodium were seen between manufacturing methods, however there were no significance differences between donor age and sex groups.

Findings of this study suggest that the membrane-related storage lesion is initiated prior to the first day of storage with contributions by both blood manufacturing process and donor variability. The findings of this work highlight the importance of characterizing membrane water permeability during storage as it can be a predictor of the biophysical and chemical changes that affect the quality of stored red blood cells during hypothermic storage.

1. Introduction

Currently, red blood cell (RBC) units stored at hypothermic temperature (1–6 °C) for up to six weeks are licenced for clinical use as long as 75% of transfused RBCs remain in the circulation for 24 h and the degree of hemolysis in the bag is less than 0.8% [22,30]. These regulations are in place to ensure that the quality of products is maintained during collection, processing, and storage and to minimize the possibility of adverse reactions in patients. However, studies in the literature have reported that stored RBCs lose membrane integrity, hemoglobin, adenosine triphosphate, and 2,3-diphosphoglycerate [15,30]. These structural and biochemical alterations have been associated with cell rigidity, morphological changes, and eventually degradation in deformability-dependent factors collectively known as the storage lesion [7,15,30]. Several studies and reviews have focused on linking the storage lesion and the loss of quality and efficacy of stored RBCs to the RBC storage duration [4,5,21,31]. Various clinical cohort studies have found correlations between storage duration and the incidence of adverse transfusion outcomes, including immunomodulatory effects, increased length of stay in hospital, and organ failure, particularly in the

Abbreviations: RBC, red blood cell; CBS, Canadian Blood Services; WB, whole blood; RCF, red cell filtration method; WBF, whole blood filtration method; Lp, water permeability; CPD, citrate-phosphate-dextrose anticoagulant; SAGM, saline adenine glucose mannitol; EI_{max}, maximum elongation index; K_{EI}, 50% of the maximum elongation; MCF, mean corpuscular fragility; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration

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case of critically ill patients [14,23].

Recently, the argument has been made that in addition to RBC storage duration, blood component manufacturing methodology influences the quality of stored RBCs [1,16,26]. The two manufacturing processes used by Canadian Blood Services (CBS) to separate RBCs from whole blood (WB) collections include the red cell filtration method (RCF, top and bottom) and the whole blood filtration method (WBF, top and top) [13]. The main differences between these two methods are: the duration and hold temperature before processing, the speed and length of centrifugation, the component types which are derived from whole blood, and the stage of processing where leukoreduction occurs [1,13]. While recent studies have described significant impacts of manufacturing processes on RBC quality measures such as hemolysis, hemoglobin content, and residual plasma, the quality impacts to functional RBC membrane characteristics have not been fully explored [1,3,12].

The characteristics of the donor, or donor-to-donor variability, are an important factor which may affect the quality of stored RBCs. Donor characteristics refer to a wide range of variables present in the donor population including biological factors such as sex, age, ABO-Rh blood group, conditions such as thalassemia, or behavioral and lifestyle differences such as donation frequency, smoking, and alcohol consumption. A recent review by Tzounakas et al. looked at the potential impact of these determinants on blood donations [29]. Recent work by Jordan et al. has found that donor age and sex have a significant impact on RBCs' storage quality, including hemolysis and hematocrit [17,20]. In this paper, we used donor age and sex as determinants for donor related effects on RBCs.

The cell membrane regulates the osmotic properties, such as water permeability (Lp), of RBCs when flowing through a medium of different osmolality by maintaining the movement of water and solutes between cytoplasm and the extracellular environment until osmotic equilibrium is achieved [10,24,28]. Previously, we were able to demonstrate that Lp in RBCs changed significantly as a function of storage duration [2]. In this study, membrane water permeability and other membrane quality measures were examined as a function of RBC manufacturing methods and donor characteristics (age, sex) throughout storage. As hemolysis testing is one of the best clinical standards for assessment of RBC quality [20], multiple regression models were also applied to hemolysis data obtained at the same time as the membrane water permeability data to test how much of the variability in RBC hemolyis and water permeability can be explained by the storage length, manufacturing method, and donor characteristics.

2. Methods

2.1. Blood manufacturing

Fifty-one WB units from healthy blood volunteers were collected and processed at three CBS production sites; British Columbia & Yukon (19 units), Calgary (13 units), and Dartmouth (19 units). WB, with a target collection volume of 480 mL, were mixed with 70 mL of citrate-phosphate-dextrose (CPD) anticoagulant and either processed by RCF (n = 27) or WBF (n = 24) (Fig. 1) [1]. Briefly, RCF units were processed using the top-and-bottom system in which WB units were stored for up to 20 h at room temparature and then centrifuged at 3493× g for 11 min. Saline-adenine-glucose-mannitol (SAGM) additive solution was added to RBC units after component separation, followed by leukoreduced filtration. In WBF (top-and-top system), WB units were held in the refrigerator at 1–6 °C for up to 72 h before leukoreduction and then centrifuged at 4552× g for 6 min. SAGM was added to extracted RBCs [1,12,13]. RBC units were packed in a temperature controlled shipping container (1–10 °C) and shipped to CBS Edmonton site. All units were stored at 1–6 °C upon receipt. Three test points were used: day 5 ± 2, day 21, and day 42. At each testing point, units were gently mixed, and 5 ± 1 mL was drawn from the bag and used for *in vitro* measurements. This research protocol was reviewed and approved by the Research Ethics Boards of the University of Alberta and Canadian Blood Services.

2.2. Donor characteristics

Red blood cell units were grouped according to donor age and sex and were processed by RCF and WBF methods. Twenty-seven red blood cell units were obtained from male donors; 15 units from male donors ≥50 years of age (9 units were processed by RCF, and 6 units were processed by WBF) and 12 units from male donors ≤ 30 years of age (6 units were processed by RCF, and 6 units were processed by WBF). Twenty-four RBC units were obtained from female donors; 12 units from female donors ≥50 years of age (6 units were processed by RCF, and 6 units were processed by WBF) and 12 units from female donors ≤ 30 years of age (6 units were processed by RCF, and 6 units were processed by WBF).

2.3. Water permeability (Lp)

Water permeability (Lp) was measured at day 5 ± 2, day 21, and day 42 of storage using the intrinsic hemoglobin fluorescence intensity method, as previously described [2]. Briefly, RBCs suspensions were prepared for each unit by diluting 400 μL RBCs in 20 mL 1× PBS solution with a final osmolality of 285 ± 1 mOsmol/kg. An SX18 MV stopped-flow analyzer (Applied Photophysics, Leatherhead, UK) was used to obtain one thousand data points of RBCs intrinsic hemoglobin fluorescence intensity during a 10 s exposure period after mixing equal amounts of RBC suspension with 0.5× PBS (147.5 ± 1 mosm/kg) to reach a final concentration of 0.75× PBS (final osmolality 217 ± 1 mosm/kg). Fluorescence data were converted to volume as previously described [33]. Lp was then calculated using curve fitting in Excel based on the least-squares method [34].

2.4. In vitro quality measures

Deformability of RBCs was determined at day 5 ± 2 and day 42 by ektacytometry using a Laser-assisted Optical Rotational Cell Analyzer (LORCA, Mechatronics, the Netherlands). Outcome data was used to determine the maximum elongation index (EI_{max}) and the 50% of the maximum elongation (K_{EI}) [32]. At the three testing points of day 5 ± 2, day 21, and day 42, hemolysis was determined using Drabkin's method and a manually measured hematocrit. The percent hemolysis (%) was determined from the ratio of supernatant hemoglobin concentrations to the total hemoglobin concentrations with correction for the hematocrit [1]. The mean corpuscular fragility (MCF) was also used to assess the ability of RBCs to resist osmotic stresses in decreasing concentrations of buffered salt solution. The concentration of salt required to cause 50% hemolysis in a sample represents the MCF [25]. A SpectraMax 384 Plus spectrophotometer (Molecular Devices Corp., Sunnyvale, CA) was used for both hemolysis and MCF to measure the absorbance of hemoglobin at 540 nm which is directly related to the amount of hemoglobin in the solution. The RBCs indices including the mean corpuscular volume (MCV), the mean corpuscular hemoglobin (MCH), and the mean corpuscular hemoglobin concentration (MCHC) were also assessed at all three testing points using an automated cell counter (Beckman Coulter ACT 8, Fullerton, CA). Supernatant potassium (K^+) and supernatant sodium (Na^+) were also obtained for day 5 ± 2 and day 42 samples as described previously [1].

2.5. Statistical analysis

Statistical analysis was performed using SPSS statistical software (Version 23, IBM, NY). Results are reported as mean ± standard deviation (SD). In all of the of following statistical analysis, a p value of less than 0.05 was considered significant. One-way analysis of variance (ANOVA) with Tukey post hoc analysis used to identify significance

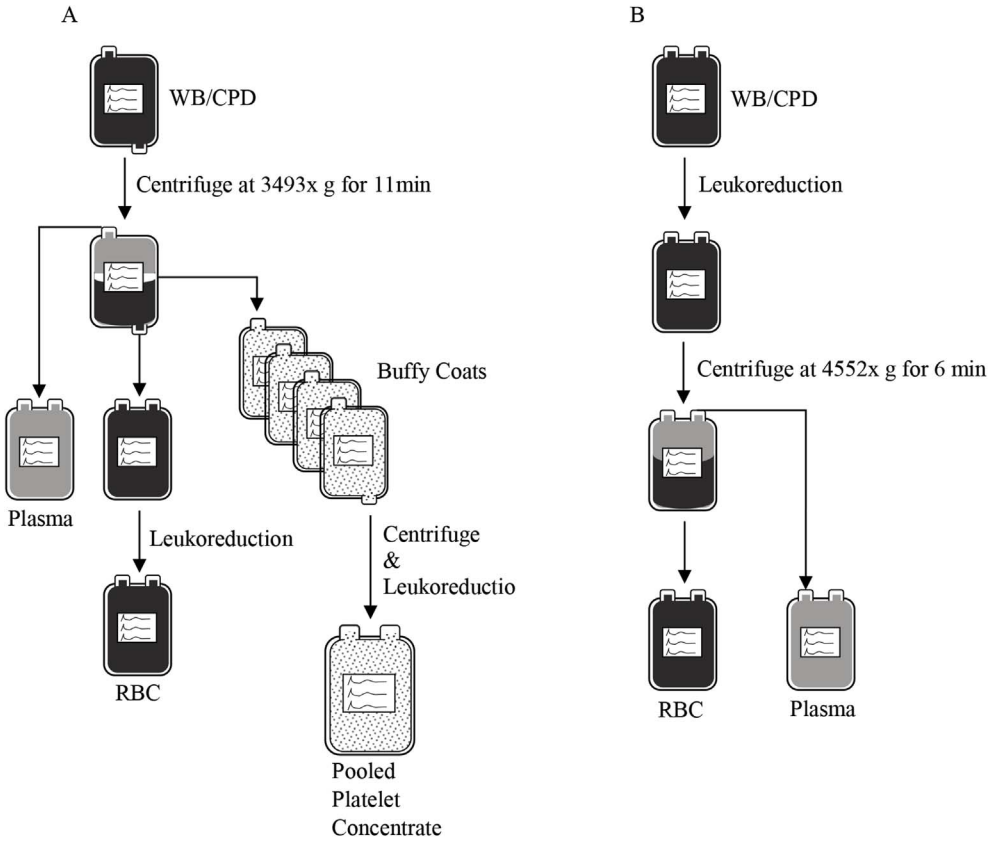

Fig. 1. Representative diagram of blood manufacturing methods of red cell filtration (RCF, top and bottom) method (A) and whole blood filtration (WBF, top and top) method (B). Collected RBCs from both methods are mixed with SAGM additive solution and stored at 1–6 °C.

among pairwise comparisons of manufacturing methods and donors age and sex group. Storage duration effects across testing time points were assessed using two-way ANOVA. Where significant differences existed between manufacturing methods, mixed between-within subjects analysis of variance (mixed ANOVA) was performed to assess the adjusted main effects and interaction effect between manufacturing process and storage duration to determine whether there were significant effects in results of RCF and/or WBF manufacturing process, and whether these results were affected by the interaction with the storage length. The type of manufacturing process (RCF vs. WBF) was considered as the between-subjects independent variable. For donor characteristics effects, donor specifications of age ($\geq$50 or $\leq$ 30) and sex (male or female) were considered the independent between-subjects' variables. In both cases, the storage durations at each testing time point (day 5 $\pm$ 2, day 21, or day 42) were considered the within-subjects' variables. Multiple regression was then conducted to provide a model to test the possible contribution of storage duration, manufacturing method, and donor characteristics on the prediction of water permeability and hemolysis.

3. Results

3.1. Impact of manufacturing on water permeability (Lp) and in vitro quality measures

RCF units had a significantly lower Lp than RBC units prepared by WBF at all three testing points (Fig. 2). At day 5 $\pm$ 2, the Lp of RCF units was 15.3 $\pm$ 4.8 µm/min/atm, which was significantly different ($p < 0.05$) than the 29.0 $\pm$ 4.7 µm/min/atm value obtained from WBF units. The significant differences between units of RCF and WBF were also observed at day 21 and day 42 where Lp was 24.3 $\pm$ 7.0 µm/min/atm and 27.9 $\pm$ 6.0 µm/min/atm, respectively, for units prepared by RCF, and 30.8 $\pm$ 5.4 µm/min/atm and 35.2 $\pm$ 5.5 µm/min/atm, respectively, for WBF units. In addition, investigating the storage duration effect revealed that the Lp of RCF units was significantly increased at day 21 and day 42 when compared to fresh testing results of day 5 $\pm$ 2 ($p < 0.05$). In contrast, the WBF Lp was not significantly affected at day 21 or day 42. The mixed ANOVA analysis indicated that there was a significant interaction effect between the manufacturing process and storage duration ($p < 0.01$); indicating that the effect of the manufacturing method on water permeability is dependent on the storage duration. Therefore, to describe the influence of unit processing, a related storage interval needs to be specified. The main effects of each independent factor (manufacturing method and storage duration) were also significant ($p < 0.01$). However, storage duration alone had a more significant main effect than the manufacturing method alone.

Deformability measurements of RCF and WBF for day 5 $\pm$ 2 and expiry testing at day 42 are shown in Eadie-Hofstee plot (Fig. 3). There were no significant differences between manufacturing methods in EI_{max} or K_{EI} at any testing time point. Units prepared by RCF had significantly decreased EI_{max} at the end of storage compared to the fresh testing ($p < 0.05$). However, the EI_{max} of WBF units and the K_{EI} of both RCF and WBF units were not significantly affected by storage. The interaction effect between manufacturing process and storage duration and the main effects of RCF and WBF processing did not significantly affect the EI_{max}. However, the main effect of the storage length on the EI_{max} was significant ($p < 0.01$).

Units processed by RCF method had significantly lower hemolysis at day 21 and day 42 compared to WBF ($p < 0.05$) (Fig. 4-A). However, there were no significant differences in hemolysis between the two processing methods at day 5 $\pm$ 2. RCF units had a significant increase in hemolysis between day 5 $\pm$ 2 and expiry ($p < 0.05$). A significant increase in hemolysis occurred earlier, by day 21, for WBF units and remained significant at day 42 ($p < 0.05$). The mixed ANOVA analysis showed that there was a significant interaction effect from storage duration and the manufacturing process ($p < 0.01$). In addition, the main effect of storage duration and manufacturing method were also significant ($p < 0.01$).

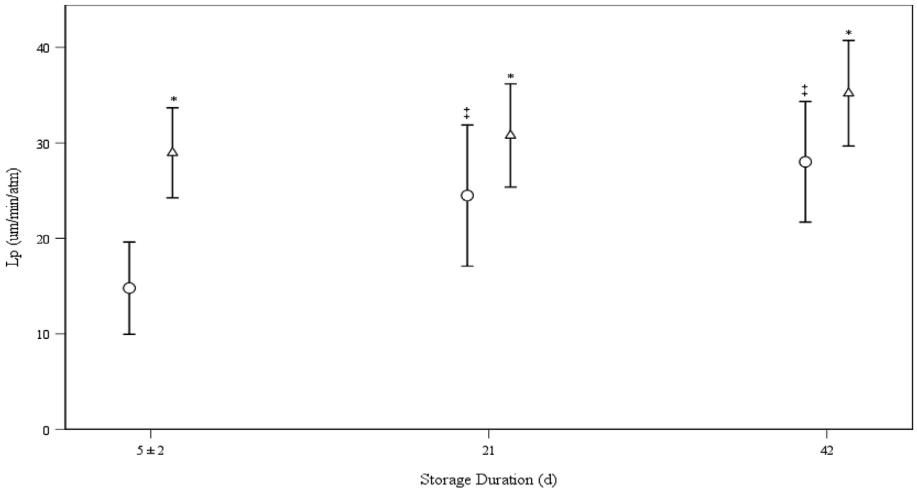

Fig. 2. Effect of blood manufacturing process on water permeability of stored RBCs. Water permeability was measured at three time points of day 5 ± 2, day 21, and day 42. The results are mean ± SD. (○) Red cell filtration method, $n_1 = 27$ (△); Whole blood filtration method, $n_2 = 24$. p values of less than 0.05 were considered to be significant.
* significant mean difference when compared with red cell filtration method at the same testing time point.
‡ significant results in comparison to fresh testing of the same manufacturing method.

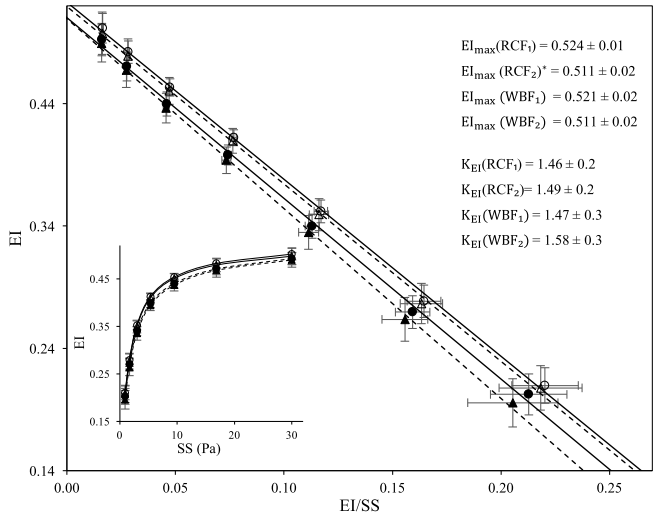

Fig. 3. Eadie-Hofstee plot for RBC deformability in red cell filtered (fresh = RCF1, expiry = RCF2) and whole blood filtered unit (fresh = WBF_1, expiry = WBF_2). Reported EImax and KEI are mean ± SD. ( ) RCF_1 (—○—), RCF_2 (·•·), WBF_1 (– ▲ –), WBF_2. EI: elongation indexes. SS: shear stress.
* significant mean difference when compared with fresh testing of the same manufacturing method ($p < .05$).

The osmotic fragility and RBC indices parameters did not show significant differences for either storage duration or manufacturing process, with the exception of MCHC (Fig. 4-B). MCHC measurements throughout storage were not significantly different for RCF units and WBF units at any of the three testing time points. In units prepared by the RCF manufacturing method, there was a significant reduction in MCHC at day 21 and day 42 when compared to day 5 ± 2 ($p < 0.05$). MCHC in WBF units was not significantly affected by the storage duration. The storage duration and manufacturing process interaction effect and the manufacturing process main effect on the MCHC were not significant. The main effect of the storage length was significant ($p < 0.01$).

The day 5 ± 2 and day 42 measurements of supernatant K^+ and supernatant Na^+ are shown in Fig. 4-C and D, respectively. Units manufactured using RCF had significantly higher supernatant K^+ and significantly lowered supernatant Na^+ at each testing points when compared to WBF units ($p < 0.05$). Supernatant K^+ was also significantly increased as a function of storage duration in both of RCF and WBF units ($p < 0.05$). Supernatant K^+ mixed ANOVA indicated no significant interaction effect with significant main effects of each of manufacturing method and storage length ($p < 0.01$). Supernatant Na^+ was significantly decreased at day 42 in both manufacturing methods ($p < 0.05$). The interaction effect and the main effects of manufacturing method and storage length were significant ($p < 0.01$).

3.2. Impact of donor characteristics on water permeability (Lp) and in vitro quality measures

Water permeability (Lp) measurements for different donor age and sex groups are shown in Fig. 5. Units obtained from ≥50-year-old male donors and processed by RCF had a significantly lower Lp than other donor groups at day 5 ± 2 ($p < 0.05$). However, there were no other significant differences in Lp between donor groups at other testing points for either RCF or WBF units. The Lp in RCF units from ≥50-year-old males was significantly increased at day 21 and day 42 when compared to the fresh testing of day 5 ± 2. The Lp of RCF units collected from other donor groups did not show a significant change between day 5 ± 2 and day 21, but significantly increased between day 5 ± 2 and day 42 ($p < 0.05$). The ≥50-year-old female group was the only age/sex group from WBF units to show a significant change in Lp between day 5 ± 2 and day 42 testing ($p < 0.05$). The mixed ANOVA results showed that there was a significant interaction effect between donor age and sex and storage duration for RCF units ($p < 0.05$), but to a non-significant degree for WBF units. The main effects of age and sex were non-significant in both RCF and WBF units. In addition, the main effect of storage duration was significant in both RCF and WBF units ($p < 0.01$).

Membrane deformability parameters of EI_{max} and K_{EI}, showed no significant differences between donor groups. The value of EI_{max} was significantly reduced at the end of testing for ≤30-year-old female units prepared through RCF ($p < 0.05$). The mixed ANOVA for EI_{max} revealed that the mean effect of storage duration was significant for all donor groups ($p < 0.01$). No significant effects were seen for the interaction between age, sex, and storage duration, or the mean effects of age and sex.

Analysis of hemolysis results revealed that there were no significant differences between age and sex groups for either RCF or WBF units (Fig. 6). However, all donor groups showed significant increases in RBC hemolysis between day 5 ± 2 and day 42 ($p < 0.05$). The age, sex, and storage length interaction effect and the main effects of age and sex were not significant for hemolysis in RCF or WBF units. The main effect of storage duration on hemolysis was significant ($p < 0.01$).

Osmotic fragility and hematologic indices (MCV, MCHC, MCH) indicated no significant differences between age and sex groups in either RCF or WBF units. MCV and MCH were not significantly affected by the storage. MCHC analysis significantly decreased between day 5 ± 2 and both day 21 and day 42 for all groups ($p < 0.05$). The mixed ANOVA

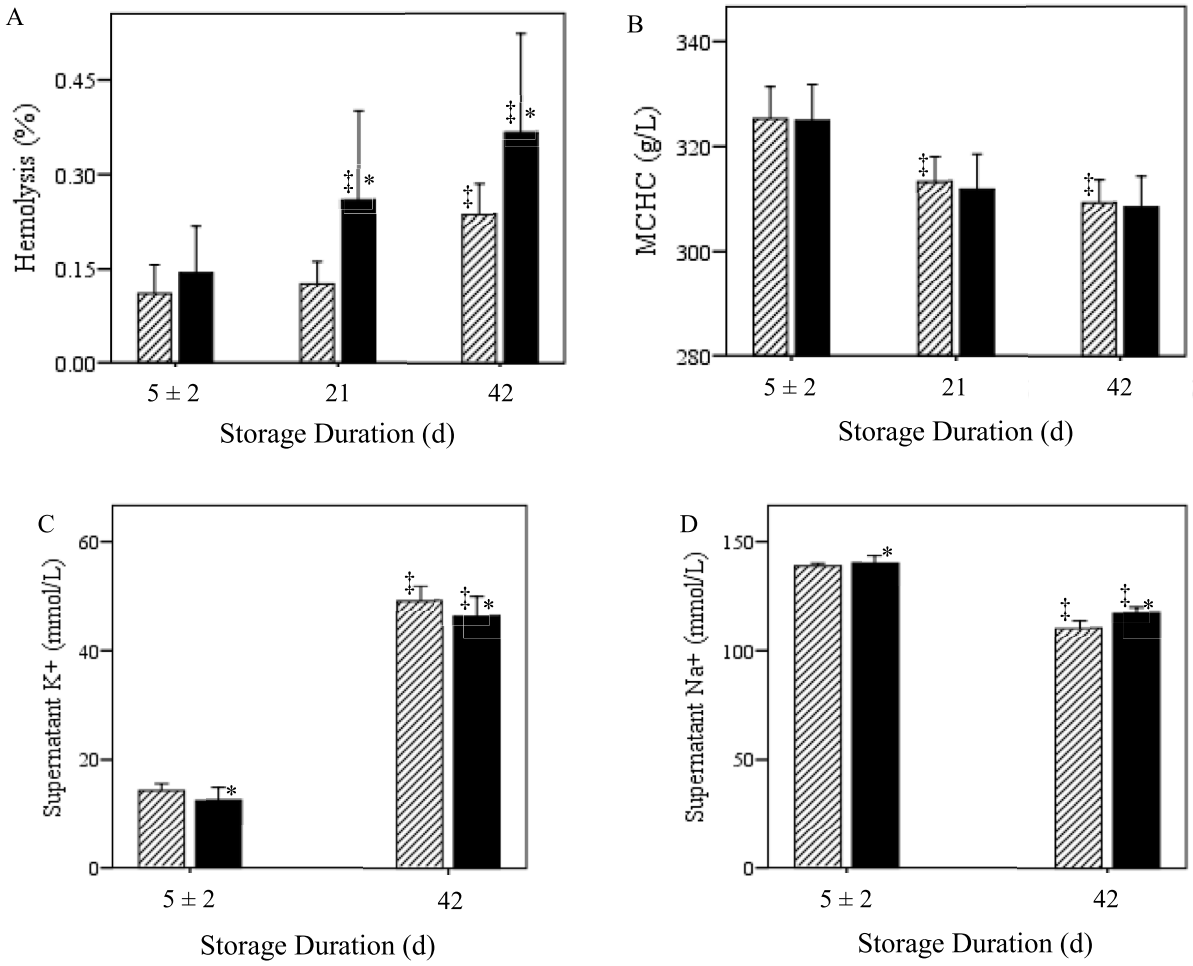

Fig. 4. Significant membrane quality differences between red cell filtration (RCF) and whole blood filtration method (WBF) units. Hemolysis (A), MCHC (B), supernatant K+ (C), supernatant Na+ (D), were measured for day 5 ± 2 (fresh testing), day 21 (middle testing),if applicable, and day 42 (expiry testing) (▨).RCF (■);WBF.

* significant mean difference between RCF and WBF units at the same testing time point.

‡ significant results in comparison to fresh testing (day 5 ± 2) of the same manufacturing method.

for MCHC revealed that the interaction between age, sex, and storage duration and the mean effect of age were not significant. The mean effect of sex on MCHC was significant only for units prepared by the RCF method ($p < 0.05$). The mean effect of storage duration on MCHC was significant for all donor groups ($p < 0.01$).

The day 5 ± 2 and day 42 measurements of the supernatant K^+ and supernatant Na^+ revealed that the concentration of supernatant K^+ for ≤30-year-old female group prepared by RCF was significantly lower than other donor groups ($p < 0.05$). No other significant differences were seen in supernatant K^+ or supernatant Na^+ between donor groups in either RCF or WBF units. Supernatant K^+ significantly increased as a function of storage duration for all donor groups ($p < 0.05$). Supernatant Na^+, however, was significantly decreased on day 42 ($p < 0.05$). The interaction effect between age, sex, and storage duration on supernatant K^+ and supernatant Na^+ was not significant. The main effect of storage duration on supernatant K^+ and supernatant Na^+ was significant ($p < 0.01$). The main effect of sex on supernatant K^+ for groups prepared by RCF was significant ($p < 0.05$). No other significant main effects were detected.

3.3. Regression model

Results of regression analysis provided a confirmation that three factors (storage length, manufacturing process, and donor characteristics) significantly contribute to variability in water permeability (Lp) and hemolysis. 53.1% of the Lp model can be predicted by measuring these three factors ($R^2 = 0.531$, $p < 0.001$). The evaluation of the contribution of each of the three factors revealed that storage length ($\beta = 0.470$) and manufacturing process ($\beta = 0.549$) make a statistically significant ($p < 0.001$) contribution to the model. The donors' age ($\beta = 0.056$) and sex ($\beta = -0.085$) do not make a significant contribution in prediction of Lp. Similarly, the hemolysis regression model can predict 48.1% of the measurement variabilities ($R^2 = 0.481$, $p < 0.001$). Storage duration ($\beta = 0.544$) and manufacturing methods ($\beta = 0.402$) have a statistically significant effect ($p < 0.001$) on the prediction of the hemolysis model. The beta value of donor's sex ($\beta = 0.130$) indicated that it had a lesser, but significant ($p < 0.05$), contribution. The contribution of donor's age ($\beta = -0.067$) was not significant in the model.

4. Discussion

There are several studies which have focused on identifying factors which cause stored RBCs to undergo the storage lesion. The vast majority of these studies have primarily evaluated the role of storage duration on the storage lesion. However, a gradual awareness of other potential factors, such as blood manufacturing methodology or donor characteristics, influencing the quality of RBCs during hypothermic storage has emeged [1,17].

The results of the present study confirm pre-existing findings which

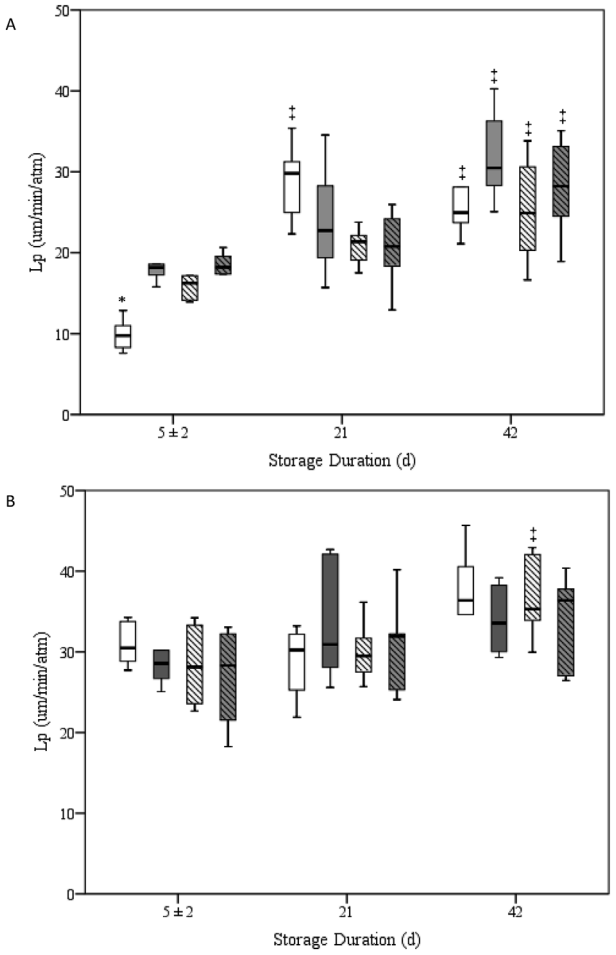

Fig. 5. Water permeability (Lp) testing for donor groups at day 5 ± 2, day 21, and day 42 for units prepared using red cell filtration (A) or whole blood filtration (B). Boxes represents the first and third quartiles of the Lp data for > 50-year-old male (□), < 30-year-old male (▩), > 50-year-old female (▨), and < 30-year-old female (▩). The band inside each box is the median, and the top and bottom of the whiskers represent the full range of the minimum and maximum limit of all of the data. p values of less than 0.05 was considered to be significant.

* significant mean difference when compared to other age/sex groups at the same testing time point.

‡ significant results in comparison to fresh testing of the same age and sex group.

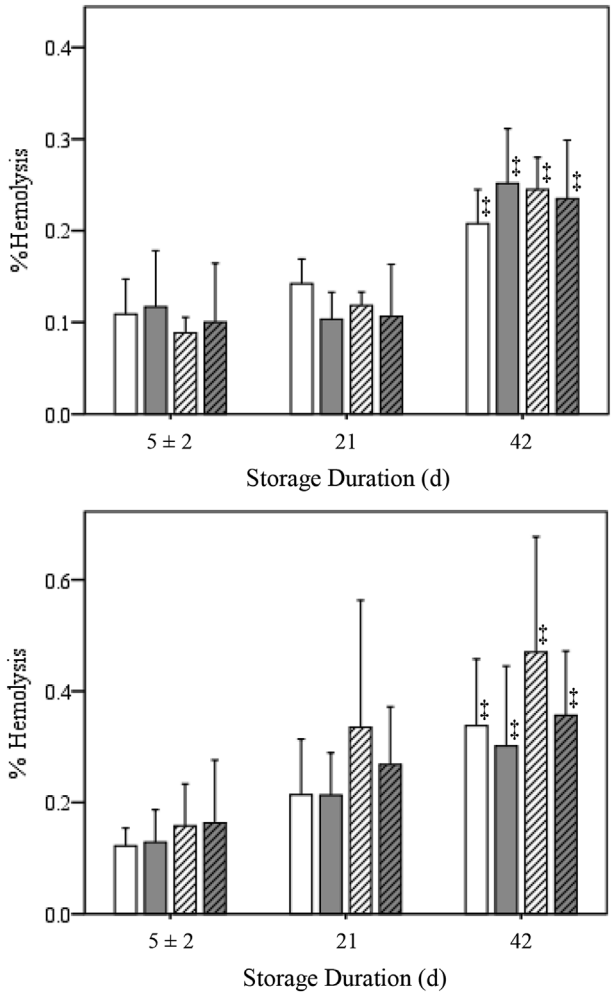

Fig. 6. Impact of donor age and sex on hemolysis in units prepared using either from red cell filtration (A) or whole blood filtration (B) at fresh testing (day 5 ± 2), middle testing (day 21), and expiry testing (day 42). Bars represent % hemolysis measures for > 50-year-old male (□) < 30-year-old male (▩) > 50-year-old female (▨) and < 30-year-old female (▩).

‡ significant results in comparison to fresh testing of the same age and sex group.

suggest that the quality of RBCs is influenced by both the manufacturing method (RCF vs. WBF) and storage duration [1,12,17]. RBC units manufactured by WBF had significantly higher Lp at all of three testing points compared to RCF units. The most striking result to emerge from the Lp data is that the value of Lp in fresh WBF units exceeded the value of Lp in expiry RCF units. Therefore, it is not surprising that manufacturing methodology has previously been shown to impact other membrane related parameters such as hemolysis [12,17] and supernatant K^+ [1], findings that were also observed in this study. We have demonstrated that the storage duration and manufacturing method have a significant main effect on hemolysis, supernatant K^+, and supernatant Na^+. These findings infer that part of the membrane-related storage lesion is initiated during RBC unit manufacturing, before the start of the post-production hypothermic storage, with the WBF manufacturing method leading to an increased amount of membrane damage in stored RBCs. However, despite this damage, some commonly used membrane related quality parameters were not able to detect differences between the two methods (EI_{max} and K_{EI}).

Various studies in the literature have also provided evidence that RBC product quality varies between manufacturing methods. A recent study by Jordan et al. (2016) examined quality control data of over 28,000 RBC units produced by RCF and WBF methods [17]. They found that compared to the WBF units, the RCF units exhibited lower hemolysis at expiry (day 43). Another major study undertaken by Acker et al. (2014) compared RBC quality monitoring data between processing methods [1]. Units prepared by WBF method were found to exhibit higher hemolysis, hemoglobin content, and supernatant K^+ and lower ATP and 2,3-DPG compared to those processed by RCF method. Hansen et al. (2015) compared the *in vitro* quality parameters of RBC units produced by nine different manufacturing methods including the two methods (RCF and WBF) included in this study [12]. They reported that RCF units exhibited the lowest hemolysis among all other methods at both fresh and expiry testing points. They also demonstrated that there were no significant differences in EI_{max} and K_{EI} between RCF and WBF methods, which is in agreement with our findings. Although there are significant differences in most *in vitro* RBC quality measures when the WBF and RCF methods are compared, these results should be interpreted with caution as these differences are not necessarily clinically significant. Future studies on the post-transfusion influences of RBCs prepared by different manufacturing methods are therefore recommended.

Contrary to what was previously reported in the literature, no significant differences were seen between units from different donor age and sex groups with the exception of Lp in fresh units and supernatant K

+ in RCF units at both fresh and expiry. While age had a non-significant impact on all parameters, mixed ANOVA showed that donor sex has a significant effect on MCHC and supernatant K^+ for units prepared by the RCF method.

Both Jordan et al. and Kanias et al. examined the impact of donor variation on quality control data from more than 16,000 male and 11,000 female CBS whole blood donors [17,20] and reported that both sex and age had significant impact on expiry hemolysis levels. The current study was unable to detect similar differences between groups, likely due to the small sample size which increased the risk of accepting a false null hypothesis (type II statistical error) [11]. It is also possible that the current study was unable to find significant differences between age groups due to the dichotomous entry of the age variable (the $\geq$50 years old category was assigned 0 and the $<$ 30 years old category was assigned 1). Kanias et al. entered their variables as a continuous interval scale [20] due to the large sample size. The current study is also subject to additional limitations. One confounding variable which may have affected membrane quality was that units were collected and processed across three different production sites which may have introduced some effect due to the shipment of the products across the country. Another limiting factor is that the fresh testing was performed at day 5 ± 2, but the likelihood of detecting differences cause by manufacturing effects may increase if all fresh testing had been performed earlier.

When looking to the literature, the impact of donor variation on RBC quality is still a highly debated topic. Daly et al. in his recent study had tested the effects of donor sex of 12 males and 12 females on stored RBCs [9]. They showed that there were no significant rheological differences between RBCs during storage which is in agreement with the current findings. The majority of previous reports, however, report quality differences due to donor factors. For example, it has been reported that stored female RBCs exhibit lower hematocrit [6,17] and mechanical fragility [27] compared to stored male RBCs. These studies suggested that female donor-derived RBCs have a more intact cell membrane and are less susceptible to storage. Male donor derived RBCs, on the other hand, have been reported to exhibit significantly lower deformability and higher fragility compared to female donor derived RBCs [22]. The variations between RBCs derived from male and female donors may be due to differences in the ratio of young to old RBCs between groups, with females having a higher ratio of young RBCs due to the regular loss of blood during menstruation [18,19]. Collectively, these observations may support the hypothesis of the existence of considerable donor sex-dependent differences which may impact RBCs quality during hypothermic storage.

It has also been demonstrated that donor age may influence the average ratio of young to old RBCs in the circulation [19]. The average age of RBCs was reported to be 50.7 ± 7.2 days (Mean ± SD) at the time of donation; approximately midway through the RBC 120 day lifespan [8]. Older males and females were found to possess more old RBCs and less young RBCs when compared to younger participants. It is hypothesized that this may relate to the declining ability of the bone marrow to generate new RBCs through erythropoiesis as a person ages [18,19]. During hypothermic storage, donor age has been associated with the level of hemolysis in the bag with younger male and female donors tending to have less hemolysis [20]. Thus, even though we did not detect any significant donor age effects on membrane quality of stored RBCs, a number of lines of evidence in the literature suggest that donor biological variation, such as age and sex, is a confounding factor that needs to be taken into account when investigating the quality of stored RBCs.

Results of the regression analysis seem to be consistent with those of previous findings on the effects of storage length, manufacturing methods, and donor characteristics. Both storage duration and manufacturing process were found to have a significant contribution to both Lp and hemolysis models. Donor sex made a significant contribution to the hemolysis model but not to the Lp model. However, the age was not considered a significant predictor in either model. The result of the hemolysis model seem to contradict the ANOVA findings, as the sex factor gave significant contribution with the hemolysis regression model but not with mixed ANOVA. A potential explanation being that a link exists between storage lesion and sex-related variations, but that the same is not true for age-related variations.

In conclusion, this study has found that water permeability can be used along with other membrane quality parameters to assess RBCs quality during hypothermic storage. Despite the small sample size, the results also support the conclusion that, in addition to storage duration, manufacturing method has an effect on RBC product quality. As significant differences in product quality were seen due to donor sex, but not age, it is likely that donor age plays less of a role in RBC quality variation during storage than donor sex. Therefore, future efforts to determine how donor factors impact quality should concentrate on the role of donor sex and related factors such as frequency of donation, or the relationship of hemoglobin donor deferral criteria on the quality of stored RBC.

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Conflicts of interest

The authors have no conflicts of interest to disclose.

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