Title:

Expediting red blood cell transfusions by syringing causes significant hemolysis

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RBC syringing causes hemolysis

Abstract:

Background

Techniques commonly used to expedite blood transfusions include pneumatically pressurizing RBC bags or manual syringing its contents. We compared these techniques on RBC hemolysis using a simulated transfusion model.

Study design and methods

Fifteen warmed 12.3 $\pm$ 4.3 (CI^{95} 10.1 to 14.5) days old RBC's were each subjected to two experimental rapid transfusion techniques. RBC's from each technique were directed through 18 and 22-gauge cannulae attached to blood administration sets. One technique involved RBC bag pressurization to 300 mmHg. The other employed a 20 ml syringe to effect forceful, manual aspiration from the RBC bag followed by forceful, manual RBC injection. The control group was gravity driven without cannulae. Free hemoglobin concentrations were measured and percentage hemolysis calculated.

Results

Free hemoglobin concentrations and % hemolysis (median; 95% CI) were similar in the control (0.05, CI^{95} 0.03 to 0.08 g/dl; 0.13, CI^{95} 0.09 to 0.17 %) and pressurized experiments (0.06, CI^{95} 0.05 to 0.09 g/dl; 0.14, CI^{95} 0.12 to 0.22 %) respectively. Syringing resulted in tenfold higher free hemoglobin concentrations (0.55, CI^{95} 0.38 to 0.92 g/dl) and % hemolysis (1.28, CI^{95} 1.03 to 2.15 %) than when employing the control ($p<0.0001$) or pressurization ($p<0.0001$) techniques. Cannula sizes studied did not affect hemolysis.

Conclusion

Forceful manual syringing caused significant hemolysis and high free hemoglobin concentrations. Pressurizing RBC bags induced no more hemolysis than following gravity facilitated transfusions. Syringing to expedite RBC transfusions should be avoided in favor of pneumatic RBC bag pressurization.

Keywords:

Transfusion rate, Red blood cells, Hemolysis, Syringing

Introduction

During resuscitation, medical personnel frequently need to expedite red blood cell concentrate (RBC) transfusions. RBC infusion rates are typically accelerated by increasing the diameter of, or pressure gradient over, the intravenous cannula. Increasing the proximal pressure is typically achieved by applying external pressure to the RBC bag. Alternatively, RBC contents may be aspirated into a syringe, which is then used to forcefully inject the blood into the patient. The latter may sometimes be the only option, for example when re-infusing cell saver blood where pressurizing the bag carries the risk of air embolism. In recent years at our hospital, manual syringing has been widely adopted even for expediting transfusions. Little is known about the effects of syringing or pressurization on red cell lysis.[1,2] We decided to compare the effects of these techniques on RBC hemolysis using a simulated expedited transfusion model.

Materials and Methods

We designed a laboratory based, paired, controlled study to compare simulated rapid transfusion techniques on RBC hemolysis. Our null hypothesis stated that there would be no difference in hemolysis when syringing RBC's or pressurizing RBC bags. The primary end points for evaluating hemolysis were free hemoglobin concentrations and the percentage hemolysis produced.

Prior ethical approval was obtained from both the local Health Research Ethics Committee (15/02/044) and also the local Blood Bank Ethics Committee. We purchased fifteen unexpired, standard quality and age CPD/SAGM (citrate-phosphate-dextrose/saline-adenine-glucose-mannitol preservation solution) RBC's from our local Western Province Blood Transfusion Service. Before the experiments, units were warmed for 60 minutes in a thermostatically controlled fluid warming cupboard set to temperature 42^{o}C. Immediately after removing each unit from this cupboard, the temperature was measured using a digital thermometer (Clicks Group Limited, Cape Town, South Africa) by inserting the tip through the RBC unit's outflow port. It was then spiked with a blood administration set containing a 160-micron filter (B Braun, South Africa, Johannesburg) and primed using gravity. To facilitate sampling, a three-way tap was attached to the end of each infusion set. Each unit was subjected to one control and four simulated expedited transfusions (Table 1). Control samples were obtained by attaching a new plastic 5 ml syringe to the three-way tap and aspirating 5 ml RBC over

approximately five seconds. Four ml was introduced into an ethylenediamine tetraacetic acid (EDTA) containing tube for free hemoglobin measurement. The rest of the sample was used to measure total hemoglobin concentration (Hb_{Total}) and hematocrit (HKT) using a HemoCue Hb 201 DM system (HemoCue AB, Angelholm, Sweden) and a GEM Premier 3500 Blood gas analyzer (Werfen Group, Barcelona, Spain) respectively.

In the S18 experiment, the three-way tap was connected to an 18-gauge intravenous cannula. The end of the cannula was directed into an empty 200 ml polyvinylchloride bag that had contained normal saline. After attaching a 20 ml syringe to the three-way tap, blood was forcefully aspirated from the RBC bag followed by forceful injection via the 18-gauge cannula into the saline bag. This was repeated three times. All aspirations and injections were performed by the same person, exerting their maximum force. The experiment was repeated using the same giving set and RBC unit, but using a 22-gauge cannula (S22 experiment), a new 20 ml syringe and a new, empty 200 ml PVC bag. After gentle mixing, a 5 ml sample was taken from the 200 ml bag contents. Sample handling and measurements were similar in the control and other experiments.

For the P18 experiment, an 18-gauge cannula was connected to the distal port of the three-way tap inserted into an empty 200 ml PVC bag. A pressure bag (VBM Infusor 500, Sulz, Germany) was wrapped around the RBC unit and inflated to 300 mm Hg. The bag pressure was transduced with a calibrated electronic pressure transducer (Biometrix, Gronsveld, Netherlands), and was kept constant while the blood ran into the saline bag. After approximately 60 ml was collected in the PVC bag, samples were collected as described above. The process was repeated using a 22-gauge cannula (P22 experiment).

The EDTA samples were centrifuged on the same day (4000 rpm, 18 ^{o}C, 5 minutes) (Beckman Coulter 22 R Centrifuge, Beckman Coulter, Fullerton, USA) to separate the supernatant from the cells. A HemoCue Plasma/ Low Hb System (Hemocue AB, Angelholm, Sweden) was used to measure supernatant free-hemoglobin concentrations (Hb_{Free}). This information was used to calculate the amount of hemolysis (Equation 1).[1]

$$Hemolysis\ \% = \frac{(100 - Hematocrit).Hb_{Free}}{Hb_{Total}} \qquad \text{Equation 1}$$

MedCalc Statistical Software version 15.11.3 (MedCalc Software, Ostend, Belgium; https://www.medcalc.org; 2015) was used for statistical analysis. Sample size calculation was based on Frelich and Ellis's findings and on finding a between-technique mean free hemoglobin concentration difference of 0.04 g/dl with a standard deviation of 0.035 g/dl.[3] A Bonferroni penalty was applied to compensate for a multiple (10) comparisons, $p < 0.05$ divided by ten indicated $p < 0.005$ would be required. The power analysis indicated that 13 units of blood would need to be studied to have a 90% power for obtaining an alpha < 0.05; however, 15 units were used to increase robustness. We considered an increase in free hemoglobin concentration of 0.1 g/dl or greater to be clinically significant.[4–6]

Results

The experiment comprised 15 control and 60 rapid transfusion simulations. RBC unit age (mean $\pm$ S.D. (95% confidence intervals)) was 12.3 $\pm$ 4.3 (10.1 to 14.5) days. Ten RBC units were group A+ and five A-. The units' temperature was 35.4 $\pm$ 0.5 (34.4 to 36.4) ^{o}C. Hematocrit was 52.1 $\pm$ 2.8 (50.5 to 53.6)%. With respect to indices of RBC lysis, variances differed and data was skewed in the S18 experiment. Therefore, non-parametric statistical analysis (Friedman Repeated Measures ANOVA) was employed. This revealed the existence of between group differences in both hemoglobin concentration and percentage hemolysis ($p < 0.00001$). Post hoc multiple stepwise comparisons identified the locations of these differences.[7] The between group indices of RBC lysis are presented in Table 2, and Figures 1 and 2.

RBC lysis, as indicated by either free hemoglobin concentrations or percentage hemolysis did not differ between the control, P18 and P22 experiments. However, statistically and clinically significantly more RBC lysis occurred in both the S18 and S22 than in the Control or Pressure experiments ($p<0.00001$) (Tables 2). Cannula size did not affect the results.

Discussion

We compared how accelerating packed red cell transfusions by either bag pressurization or syringing affected RBC hemolysis. Pressurizing RBC bags did not aggravate hemolysis compared to control. However, forceful, manual syringing caused 10-fold more hemolysis than RBC bag pressurization or that seen in the control

experiments. Cannula size did not affect the results. Our null hypothesis, that the different techniques would not differ in their propensity to cause hemolysis, was therefore rejected.

The hemolysis caused by facilitating transfusions has been investigated before. RBC age may play a role. In this respect, Miller & Schlueter observed that storage duration was the single most important factor influencing RBC lysis when subjected to syringing.[2] Red blood cell fragility increases with age, the storage period being directly related to storage medium free hemoglobin concentrations.[8,9] RBC age was unlikely to be a factor in our study as the unit we studied was relatively uniform of age, within accepted limits, USA RBC's being stored for an average of 17.9 days.[8] External pressurization of RBC bags appears safe. Mateer et al observed no greater hemolysis when transfusing from RBC bags pneumatically pressurized to 600 mmHg compared to gravity driven transfusions.[10] Similarly, Frelich and Ellis demonstrated that with respect to hemolysis, facilitating rapid transfusions by pneumatically pressurizing (150 to 300 mm Hg) RBC bags was safe.[3] Our results concur, no significant hemolysis occurring after pressurizing RBC's to 300 mm Hg. The use of 22-gauge or larger cannulae does not appear to aggravate hemolysis. Miller & Schlueter studied hemolysis resulting from forcefully syringing blood through different sized cannulae.[2] When 22-gauge or larger cannulae were used, cannula size played no role in RBC lysis. Similarly, our study indicated cannula size (admittedly only 18- and 22-gauge cannulae) did not influence hemolysis. However, Miller & Schlueter demonstrated progressively more hemolysis when 23 gauge or smaller cannulae were employed for rapid transfusion.[2] Our study uniquely compared the hemolytic consequences of RBC bag pressurization with forceful, manual syringing. Only forceful syringing caused significant hemolysis. This differs from Miller & Schlueter's study where syringing did not cause significant hemolysis. Both their and our studies employed maximum force, manual injection to enhance transfusion rates. The difference may be that Miller & Schlueter gently aspirated 1 ml aliquots out of the RBC bag, whereas we forcefully aspirated RBC's into the syringe. We hypothesize that the manual, forced aspiration greatly increased RBC shear stress and hemolysis.[1,4,11] In our study, RBC units were warmed in controlled environment to 35.4 $\pm$ 0.51 (95% CI 34.4 to 36.4) ^{o}C. Overheating can cause hemolysis, but only if red cell concentrate temperatures exceed 46°C, an unlikely occurrence in our study.[4]

The spectrin-protein cytoskeleton and red cell membrane lipid bilayers resist RBC shear stress-induced deformation. The magnitude of shear stress determines whether RBC's undergo reversible deformation,

damage, or lysis.[12] Shear stresses experienced during trans-capillary passage cause reversible, non-damaging RBC deformation but stresses exceeding 150 Pa cause permanent RBC deformation.[12,13] When shear stress exceeds normal physiological values by a factor of 10, RBC membrane integrity is compromised and hemolysis occurs.[1,12] The magnitude of shear stress is proportional to viscosity and velocity. RBC concentrate has a high viscosity and facilitating RBC transfusion expressly increases RBC velocity.[1] At high flow velocities, flow patterns change from laminar to turbulent. Turbulent flow creates vortices which greatly increase surface contact, thereby increasing shear stress.[1,12] We suspect that in our study, forceful aspiration and injection both played a role in aggravating shear stress.

Hemolysis with increased free plasma hemoglobin is an increasingly conspicuous suspect in transfusion related, adverse patient outcomes.[9,14] Free hemoglobin is cleared by binding to haptoglobin, this capacity being saturated at free hemoglobin concentrations between 0.07 and 0.15 g/dl.[1,15,16] At higher free hemoglobin concentrations, the aggravated nitric oxide scavenging promotes vasoconstriction, impairs microcirculatory flow, and causes increased pulmonary and systemic blood pressures.[9,14,17,18] This increased afterload, and the accompanying impairment of coronary blood flow cause deterioration in left ventricular performance.[18–20] Nitric oxide scavenging also promotes systemic inflammation and a pro-thrombotic state.[4,9,14,15,17,21,22] Glomerular hemoglobin filtration may cause renal injury due to both iron-facilitated generation of reactive oxygen species and tubular obstruction.[23] Cardiac surgical patients in particular suffer haptoglobin depletion, making them particularly sensitive to the deleterious effects of free hemoglobin.[9]

Blood transfusion services define significant hemolysis as being free hemoglobin concentrations above 0.1 g/dl and hemolysis ≥ 1 %. At these levels, clinically significant adverse effects appear.[4,10,24] Healthy individuals were asymptomatic following transfusion with blood having free hemoglobin concentrations between 0.2 g/dl and 1.0 g/dl.[25] Free plasma hemoglobin concentrations exceeding 0.064 g/dl has been associated with acute kidney injury following major surgery.[6] The risk for acute kidney injury increased fivefold when free hemoglobin exceeded 0.1 g/dl during cardiopulmonary bypass.[16] Plasma hemoglobin levels exceeding 0.05 g/dl were related to higher mortality in extracorporeal membrane oxygenation (ECMO) patients.[5] In the presence of left

ventricular assistant devices, free hemoglobin levels exceeding 0.04 g/dl was associated with 4 and 15-fold greater risks of death and adverse events resepctively.[22]

Our syringing experiment induced severe enough hemolysis (free hemoglobin 0.53 g/dl) that it would likely cause excessive free hemoglobin concentrations during an actual transfusion. We estimated that syringing three RBC's to a 52 kg patient would deliver 55 mg/kg of free hemoglobin, if assuming RBC volumes of 360 ml and a hematocrit of 52%. Administration of 10 mg/kg of free hemoglobin significantly increased cytokine concentrations and 15 mg/kg caused multi-organ dysfunction in pigs.[26] Syringing just one RBC to a 10 kg child would be estimated to administer double the aforementioned dose of free hemoglobin.

The maximum force exerted on the syringe was not measured, but the method used represents observed clinical practice. We did not study the effects of different aged RBC's or smaller than 22-gauge cannulae. We also did not separate the effects of forceful aspiration and forceful injection, this requiring further research. This was also a laboratory study. Clinical consequences could potentially be aggravated by concomitant haptoglobin depletion, organ dysfunction or existing hemolysis as with cell saver usage and prolonged cardiopulmonary bypass. This would represent an area for further research.

In conclusion, we compared syringing and pressurizing RBC's to expedite RBC transfusions. Our results indicate that syringing using both forced aspiration and injection causes tenfold more hemolysis than RBC pressurization. The degree of hemolysis was significant, with extremely high free hemoglobin concentrations measured in the syringed blood. While syringing of a single RBC unit would be unlikely to cause clinically significant deleterious effects in an otherwise healthy adult, the consequences may become significant during massive transfusions or in vulnerable patients. Syringing to expedite RBC transfusions should be avoided in favor of RBC pressurization.

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Tables:

Table 1: Description of experiments

Experiments	Description
Control C	Transfusion set alone, no cannula, gravity primed
S18	20 ml syringe, 18 gauge intravenous cannula
S22	20 ml syringe, 22 gauge intravenous cannula
P18	Pressure bag 300 mmHg, 18 gauge intravenous cannula
P22	Pressure bag 300 mmHg, 22 gauge intravenous cannula

Table 2: Hemolysis induced by facilitated RBC transfusion

	Free Haemoglobin concentrations [g/dl]		Hemolysis expressed as a percentage	
	Median [IQR]	**95% CI**	**Median [IQR]**	**95% CI**
Control	0.05 [0.03-0.08]	0.03-0.08	0.13 [0.09-0.17]	0.09-0.17
S18	0.55 [0.28-0.82]	0.38-0.92	1.28 [0.72-1.84]	1.03-2.15
S22	0.50 [0.37-0.63]	0.33-0.59	1.13 [0.75-1.51]	0.71-1.47
P18	0.05 [0.03-0.07]	0.04-0.08	0.12 [0.07-0.17]	0.09-0.19
P22	0.06 [0.04-0.08]	0.05-0.09	0.14 [0.09-0.20]	0.12-0.22

IQR: interquartile range (25 % and 75%), 95% CI: 95% confidence intervals. See Table 1 for explanation of experiments

Higher free hemoglobin concentrations and greater amounts of hemolysis were observed in the S18 and S22 experiments than in the control, P18 and P22 experiments ($p<0,05$). We refer the reader back to the text for the statistical methods used.

Figures:

Figure 1:

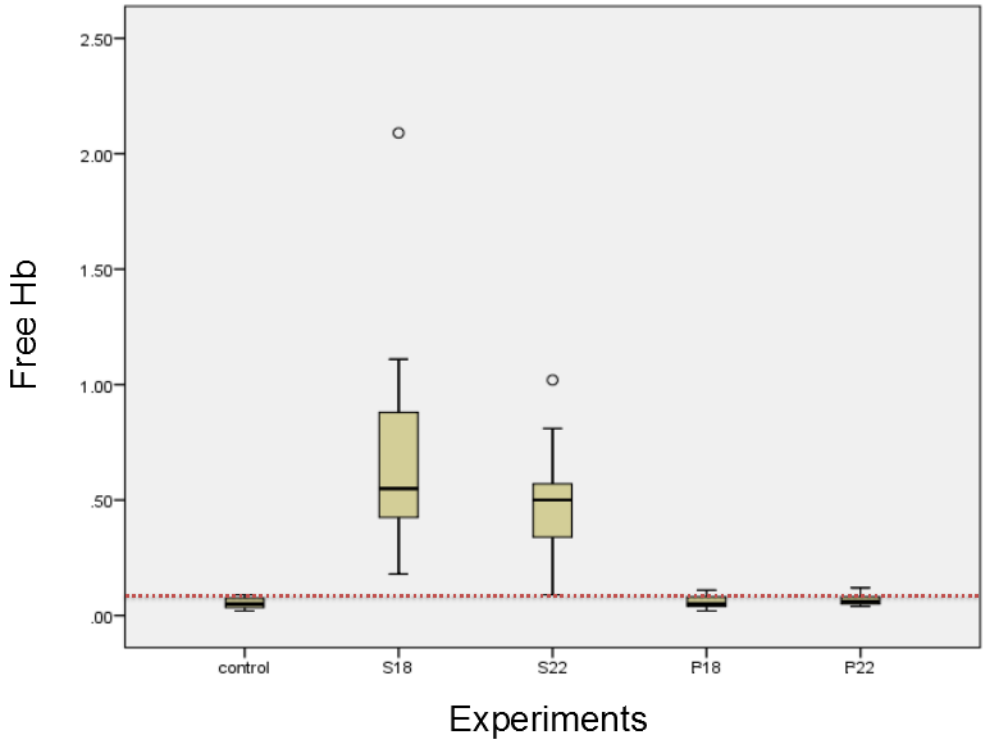
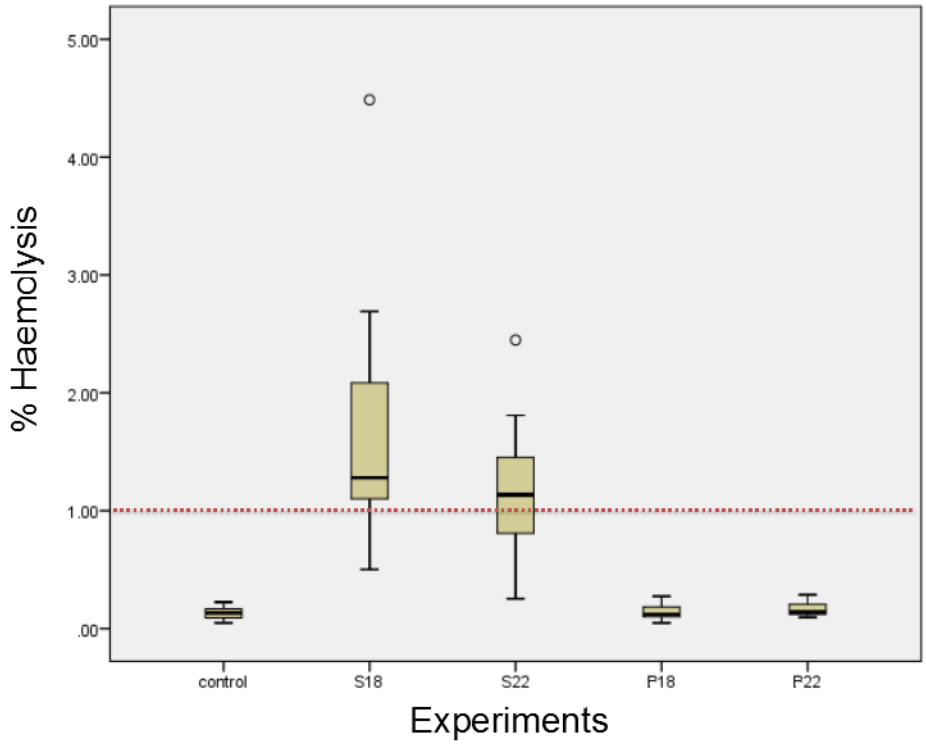


Figure 2:



Legends of figures:

Figures 1 and 2: Box and whisker plots for Free Hemoglobin and Percentage Hemolysis respectively

The red dotted lines indicate the acceptable upper values of free hemoglobin and percentage hemolysis respectively. The data is the same as that described in Table 2. Higher free hemoglobin concentrations and greater amounts of hemolysis were observed in the S18 and S22 experiments than in the control, P18 and P22 experiments ($p<0,05$). We refer the reader back to the text for the statistical methods used.