

Effects of Intraosseous Transfusion of Whole Blood on Hemolysis and Transfusion Time in a Swine Model of Hemorrhagic Shock: A Pilot Study

James M. Burgert, CRNA, DNAP

CPT John Mozer, BSN, RN, ANC, USA

CPT Tina Williams, BSN, RN, ANC, USA

MAJ Jerry Gostnell, BSN, RN, ANC, USA

Brian T. Gegel, CRNA, DNAP

Sabine Johnson, MS

MAJ Michael Bentley, CRNA, PhD, ANC, USA

Arthur "Don" Johnson, RN, PhD, Col(ret), USAFR, NC

This prospective, experimental, mixed study determined whether there were differences in intraosseous (IO) and intravenous (IV) whole blood transfusion relative to hemolysis and transfusion time. Swine were assigned to the IV group (n = 6) with an 18-gauge catheter in the auricular vein or the IO group (n = 7) with a 15-gauge IO needle in the proximal humerus. Following baseline specimen collection, 900 mL of blood was collected from each animal. The collected blood was autologously transfused by the IV or IO route using a pressure infusion bag inflated to 300 mm Hg, with immediate posttransfusion specimen collection. Hemolysis was defined by the amount of plasma free hemoglobin. Multivariate analysis of variance revealed

no significant differences between groups relative to posttransfusion free hemoglobin or transfusion time (P = .065). The IV group's mean free hemoglobin level was 10.23 ± 10.52 µmol/L; the IO group, 7.2 ± 5.82 µmol/L. The IV group's mean transfusion time was 13.48 ± 4.1 minutes; the IO group, 28.70 ± 19.51 minutes. Intraosseous transfusion does not significantly increase hemolysis or transfusion time compared with IV transfusion. Clinically, it can take up to twice as long to transfuse 900 mL of blood IO compared with IV.

Keywords: Blood transfusion, hemolysis, hemorrhagic shock, intraosseous infusion, intraosseous transfusion.

Patients in hemorrhagic shock often require transfusion of red blood cells (RBCs) to aid resuscitation and improve oxygen delivery to end organs. Obtaining vascular access in these patients remains a challenge. The intraosseous (IO) route and device may be used to transfuse RBCs when intravenous (IV) access is unattainable.

The IO route is a method to access the systemic circulation using the "noncollapsible" bone marrow matrix to deliver drugs and fluids.¹ Intraosseous devices are specialized needles used to penetrate the cortex of the bone, allowing delivery of drugs and fluids to bone marrow.² The utility and safety of IO devices as an effective means to deliver drugs, crystalloid, and colloid fluids are well established.^{3,4}

Theoretical concerns exist about the possibility of hemolysis of RBCs when administered by the IO route. Limited studies performed in pediatric swine models indicate that transfusion of RBCs through an IO device does not cause increased hemolysis.^{5,6} However, bone

marrow undergoes changes over the lifespan, resulting in decreased red-to-yellow bone marrow ratios.⁷ Age-related changes in the structure of the bone marrow may increase the rigidity of the bone marrow matrix, increasing infusion resistance and turbulent flow. Consequently, there may be an increase in shearing forces exerted on the RBC membrane, resulting in hemolysis and increased transfusion time compared with RBCs infused by the IV route.

Case reports demonstrate that IO devices have been used to transfuse blood products during emergency situations in both military and civilian practice.^{8,9} However, no studies have examined the effects of IO devices on hemolysis and time of transfusion in the adult. The purpose of this study was to determine the effect of IO vs IV transfusion of 900 mL of whole blood relative to hemolysis and time of administration. The research question that guided this study was: Are there statistically significant differences in IO and IV blood transfusion relative to free hemoglobin and transfusion time?

Materials and Methods

This study was a prospective, experimental, mixed design. The research protocol was approved by the Institutional Animal Care and Use Committee. The animals received care in compliance with the Animal Welfare Act and the *Guide for the Care and Use of Laboratory Animals*. Because this was a pilot study, the researchers did not perform a power analysis to determine the number of subjects needed. Fourteen adult male, *Sus scrofa* Yorkshire-cross swine, weighing between 67 and 80 kg, were randomly assigned by a computerized number generator to either the IV transfusion group (n = 6) or IO transfusion group (n = 7). The rationale for using this weight is that it represents the average weight of a US Army soldier. The IV group served as the control group but had 1 less subject because of clotting of blood in the collection bag. The cause of the unexpected clotting of collected blood is believed to be related to inadequate citrate in the blood collection bag. The swine were of equal size and all male to avoid any potential hormonal effects and were purchased from the same vendor from the same lot number to minimize variability.

Swine were fed a standard diet and observed for 3 days to ensure good health. The day before the experiment, the subjects were given nothing by mouth after midnight. The swine were sedated with buprenorphine (0.01 mg/kg intramuscularly [IM]) 30 minutes before anesthetic induction. Anesthesia was induced with ketamine (10 mg/kg IM) and atropine (0.05 mg/kg IM), inhaled isoflurane (4%), and oxygen (100% fraction of inspired oxygen). Isoflurane was reduced to between 1.0% and 2.0% following airway instrumentation for the remainder of the experiment. The animals were ventilated with a tidal volume of 8 to 10 mL/kg and a respiratory rate of 10 to 14/min using a Narkomed 3A anesthesia machine (Dräger Medical Systems). The swine were continuously monitored for heart rate, blood pressure, oxygen saturation, end-tidal carbon dioxide, temperature, and cardiac electrical activity via electrocardiogram (GE Marquette Solar 800 monitor, GE Healthcare). An activated clotting time (ACT) measurement was performed on each animal to rule out preexisting coagulopathy.

The left carotid artery was cannulated with a 20-gauge catheter using a cut-down technique for arterial blood pressure monitoring. An 8.5 French × 10 cm right subclavian central venous catheter (Arrow International) was inserted using the modified Seldinger technique for central venous pressure monitoring and sample collection. Intravenous access was secured using an 18-gauge IV catheter placed in the auricular vein of each animal. In each animal in the IO group, a 15-gauge × 45 mm IO needle (EZ-IO, Vidacare Corp) was inserted in the proximal humeral head of the animal with a device driver (EZ-IO) from the same manufacturer. The rationale for using an 18-gauge catheter for the IV group was that its

use is a commonly accepted blood transfusion practice. The rationale for using the 15-gauge EZ-IO needle is that it is the standard IO device used by the US military. The Committee on Tactical Combat Casualty Care guidelines of 2012 recommend an 18-gauge IV catheter and if resuscitation is required and IV access is not obtainable, to use the IO route.¹⁰

Following insertion of the EZ-IO needle, as recommended by manufacturer's instructions, placement was confirmed by aspiration of bone marrow and the ability to easily flush the device with 10 mL of 0.9% normal saline. The fasting deficit was replaced before hemorrhage using the Holliday-Segar formula. Animals were allowed to stabilize for 15 minutes before beginning the experiment.

Baseline blood specimens were collected from each animal before beginning the experiment to ensure there was no preexisting free hemoglobin. The investigators connected standard IV tubing from the central venous catheter to a blood collection bag containing citrate phosphate dextrose solution, and 900 mL of blood was exsanguinated by gravity over 15 to 20 minutes as measured by an electronic scale (Thermal Industries of Florida). The blood bags were placed on the scale and zeroed to account for variations in blood bag weight. After exsanguination, the investigators collected blood specimens from the blood collection bag and from the animal. All the samples from the animals were collected with a 10-mL syringe using the central venous catheter. Specimens collected from the blood collection bag were also collected with a 10-mL syringe using an 18-gauge needle. For the purposes of this study, hemolysis was defined as the amount of free hemoglobin. Therefore, all specimens were analyzed for free hemoglobin.

After the second specimen collection, the 900 mL of exsanguinated blood was autologously transfused into the animal from which it was exsanguinated. The blood administration system (Sangofix, Braun Medical Inc) was used for transfusion in all animals in both groups. The system is made of clear PVC tubing 150 cm in length, with a lumen measuring 3 mm × 4.1 mm, and has a drip chamber equipped with a standard blood filter of 200 µm to eliminate blood clots. An extended, detached drip element in the drip chamber provided even drop formation (20 drops = 1 mL ± 0.1 mL). The system had an elastic pump chamber, including a blood filter basket with a pore size of 200 µm and filter area of 11 cm².

The blood was transfused via the auricular IV catheter or the humeral IO route using a pneumatic pressure infusion bag inflated to 300 mm Hg. One investigator continuously monitored and adjusted the pneumatic pressure bag, ensuring that a constant 300 mm Hg of pressure was applied. Transfusion time was measured with an accurate and precise stopwatch. The final specimen collection was drawn immediately after transfusion from the animal, followed by 10 mL of 0.9% normal saline flush. All

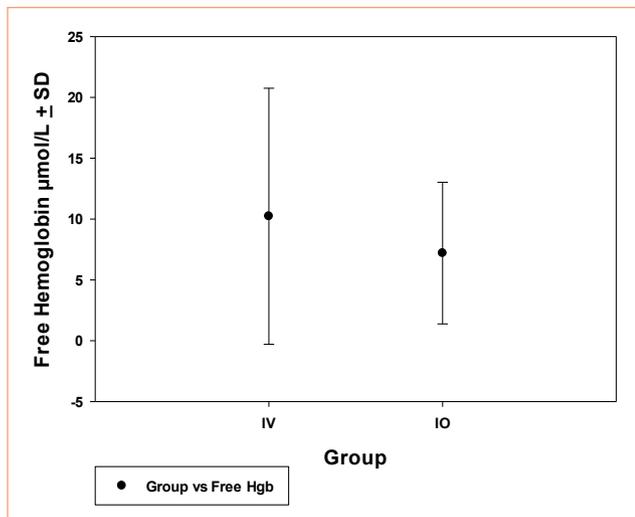


Figure 1. Free Hemoglobin Levels (mean)
Abbreviations: Hgb, hemoglobin; IO, intraosseous; IV, intravenous.

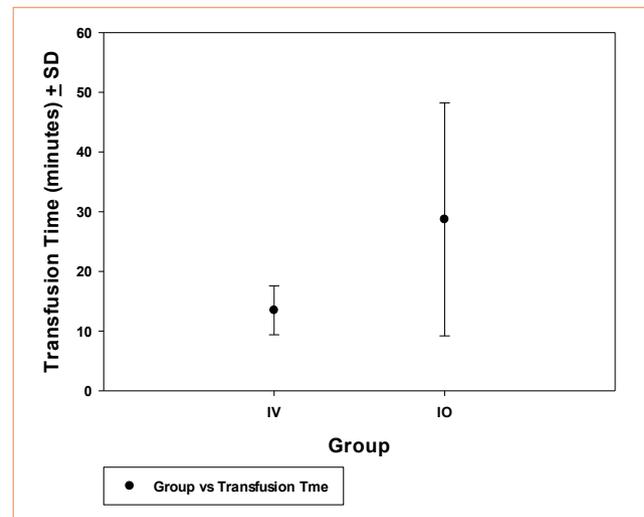


Figure 2. Transfusion Time (mean)
Abbreviations: IO, intraosseous; IV, intravenous.

specimens were collected in labeled, heparinized tubes and were centrifuged on-site. The centrifuged specimens were immediately sent to a laboratory for analysis of free hemoglobin using the plasma hemoglobin assay test. The laboratory technologist was blinded to the group assignment of the sample being analyzed.

Results

The α for this study was .05 throughout. Multivariate analysis of variance (MANOVA) found no significant differences between groups relative to pretest measures of weight, vital signs, ACT, fasting fluid deficit replacement, or free hemoglobin ($P > .05$), which indicated that the 2 groups were equivalent on these variables. All ACT results were within normal limits. The baseline free hemoglobin level from the animals and blood bags was less than 1 $\mu\text{mol/L}$. Additionally, MANOVA indicated there were no significant differences between groups relative to free hemoglobin after transfusion or transfusion time ($P = .065$). All results were expressed as the mean \pm standard deviation.

Free hemoglobin levels in the IV infusion group ranged from 8 to 27.5 $\mu\text{mol/L}$ (mean, 10.23 ± 10.52 $\mu\text{mol/L}$). The free hemoglobin levels in the IO infusion group ranged from 8 to 15.7 $\mu\text{mol/L}$ (mean, 7.2 ± 5.82 $\mu\text{mol/L}$; Figure 1). The transfusion time for the IV group ranged from 8.68 to 19 minutes (mean, 13.48 ± 4.1 minutes). The transfusion time for the IO group ranged from 8.30 to 60 minutes (mean, 28.70 ± 19.51 minutes; Figure 2).

Discussion

The theoretical framework used for this study was based on the Poiseuille law, which states that flow is related to pressure change, fluid viscosity, tubing length, and tubing radius. This law further states that radius to the

fourth power is directly proportional to blood flow and is inversely related to resistance¹¹:

$$\text{Blood Flow} = \frac{P\pi R^4}{8\eta L}$$

where P = pressure difference; R = radius; η = viscosity; and L = length.

The investigators surmised that the tortuous circulatory matrix of the bone marrow would increase resistance to infusion and shearing forces exerted on the RBC membrane, resulting in increased hemolysis and prolonged transfusion time for the IO group compared with IV group. Free hemoglobin was used as an outcome measure because it is specific to RBC hemolysis.¹²

Intraosseous transfusion of blood products in adults has been sporadically reported in the medical literature as far back as the 1930s.¹³ To the authors' knowledge, no study to date has attempted to quantify the amount of hemolysis in an adult subject transfused with RBCs via an IO device. Previous studies of IO transfusion in pediatric swine indicate there is no increase in hemolysis.^{5,6} However, adult, human red marrow concentrates toward the proximal and distal ends of the long bones while the marrow in the bone shaft changes to more adipose yellow marrow. Red marrow consists of 40% fat, 40% water, and 20% protein. Yellow marrow consists of 80% fat, 15% water, and 5% protein.⁷ The investigators theorized that the age-related changes of the bone marrow may increase resistance and turbulent flow of blood, resulting in increased hemolysis and transfusion time.

There was no statistically significant difference in the amount of hemolysis between the groups. However, as a clinical note, the mean free hemoglobin level was higher in the IV group. The reason the IV group demonstrated a higher free hemoglobin level may be related to the smaller diameter of the IV catheter (18 gauge), resulting in in-

creased resistance to infusion and subsequent hemolysis compared with the larger-bore IO needle (15 gauge).

Although there was no statistically significant difference in transfusion time, the time difference may be clinically significant. The mean transfusion time of 900 mL of whole blood was 13.5 minutes in the IV group compared with 28.7 minutes in the humeral IO group. Although the mean transfusion time of the IO group is more than double that of the IV group, an IO device can be inserted in less than 30 seconds, whereas placement of an IV catheter in a patient in shock may take considerably longer, if successful at all.¹⁴ Placement of the IO device is reported to have a higher rate of procedural success than IV or central line placement, with the potential of decreasing the time delay in initiating transfusion therapy.¹⁵

One explanation for longer transfusion times in some of the animals in the IO group may be the greater difficulty in placing an IO device in the pig humerus to achieve optimal infusion rates compared with the human humerus. Because the swine humerus is a weight-bearing bone and far denser than the human humerus, placement of an IO device requires the operator to insert the IO device into a relatively small area to obtain the fastest infusion time. Other explanations include increased resistance to infusion caused by age-related changes of the bone marrow in some animals and the inherent resistance of the bone marrow to infusion secondary to its tortuous circulatory matrix.

Internal validity of this study was maintained by minimizing variability with respect to infusion pressure, viscosity, tubing length, and tubing radius. All blood was transfused, undiluted, at 300 mm Hg through IV tubing of the same length. EZ-IO devices are standardized, with 15-gauge diameters and lengths varying from 15 mm for pediatrics, 45 mm for adults, and 65 mm for larger adults. The length of the IO device used in this study was 45 mm. The IV catheter was 18-gauge and 32 mm in length. The investigators acknowledge that possible differences in free hemoglobin and transfusion time between the groups may be the result of the IV catheters and IO devices not being the same diameter and length. However, both the IV and IO procedures used in this study are generally accepted practices in civilian and military settings. The investigators believe the differences in diameter and length of the IV catheters and IO devices selected for use in this study were negligible.

The major limitation of this study is that the results from a swine model may not be completely generalizable to humans. However, swine have very similar bone, cardiovascular, and blood physiology and serve as an excellent model for this type of research.¹⁶ Another limitation is that the use of whole blood is generally limited to military settings after stored RBCs have been depleted. This study used autologous whole blood because processing exsanguinated blood into separate components

was impractical and would have added great expense and decreased the efficiency of conducting the experimental protocol. Furthermore, refrigerated storage of packed RBCs has been associated with hemolysis. Biochemical and morphologic changes related to storage increase oxidative stress on RBCs, altering membrane integrity.¹⁷ These changes to the RBC membrane lead to alteration in shape and increased cell rigidity, increasing the possibility of hemolysis during transfusion.¹⁸ Serial measurements of free hemoglobin were not performed in this study and may be a consideration in future studies. Future studies could strengthen internal validity by using IV and IO infusion catheters as close to each other as possible with respect to diameter and length.

This study investigated the effects of whole blood transfusion via an IO device compared with an IV catheter in an adult swine model of hemorrhagic shock relative to hemolysis and transfusion time. The findings of this study demonstrate that there was no statistically significant difference between the IV and IO groups in respect to hemolysis or transfusion time. There may be clinical significance based on that it can take more than twice as long to transfuse 900 mL of whole blood IO compared with IV delivery in some cases.

Based on the findings of this pilot study, transfusion of whole blood through an IO device is an effective transfusion method that may be used until other vascular access is obtained. Furthermore, IO transfusion does not increase hemolysis of RBCs in a swine model of hemorrhagic shock. Additional investigations are needed that include a larger sample size based on power analysis, the addition of an experimental group transfused with a mechanical rapid infusion device, and expansion of the study to randomized controlled trials in humans.

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AUTHORS

James M. Burgert, CRNA, DNAP, is an associate scientist with the Geneva Foundation, Tacoma, Washington, and an adjunct assistant professor of nurse anesthesia for the US Army Graduate Program in Anesthesia Nursing, Fort Sam Houston, Texas. Email: jburgert@sbcglobal.net.

CPT John Mozer, BSN, RN, ANC, USA, is a Phase II student in the US Army Graduate Program in Anesthesia Nursing, Tripler Army Medical Center, Honolulu, Hawaii.

CPT Tina Williams, BSN, RN, ANC, USA, is a Phase II student in the US Army Graduate Program in Anesthesia Nursing, Tripler Army Medical Center.

MAJ Jerry Gostnell, BSN, RN, ANC, USA, is a Phase II student in the US Army Graduate Program in Anesthesia Nursing, Tripler Army Medical Center.

Brian T. Gegel, CRNA, DNAP, is owner of Veteran Anesthesia Services, San Antonio, Texas; an associate scientist with the Geneva Foundation; and an adjunct assistant professor of nurse anesthesia for the US Army Graduate Program in Anesthesia Nursing, Fort Sam Houston, Texas.

Sabine Johnson, MS, is a research associate with the Geneva Foundation.

MAJ Michael Bentley, CRNA, PhD, ANC, USA, is associate professor of nurse anesthesia at the US Army Graduate Program in Anesthesia Nursing, Fort Sam Houston, Texas.

Arthur "Don" Johnson, RN, PhD, Col(ret), USAFR, NC, is director of research and professor of nurse anesthesia at the US Army Graduate Program in Anesthesia Nursing, Fort Sam Houston, Texas.

ACKNOWLEDGMENTS

The investigators thank the University of Texas Health Science Center at San Antonio and the US Army Graduate Program in Anesthesia Nursing for their support of this research study.

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