Effects of Albumin Supplementation during Cardioplegia Administration: An In Vitro Analysis

Rebecca Knox, MPS, CCP;* Alfred H. Stammers, MSA, CCP;† Tunisia Ellis, MPS;* Chen Gao, MD, PhD, MPS;* Bernadette Nutter, MPS;* Hunter Holcomb, MPS, CCP;* Ryan Schmer, MPS;* Lynette M. Hock, MS*

*Division of Clinical Perfusion Education, School of Allied Health Professions, University of Nebraska Medical Center, Omaha, Nebraska
†Perfusion Services, Department of Surgery, Geisinger Medical Center, Danville, PA


Abstract: Increasing, the colloid osmotic pressure (COP) of blood cardioplegia (BCP) may reduce myocardial edema and preserve cardiac function following cardiopulmonary bypass (CPB). The purpose of this study was to quantify the effects of albumin (ALB) supplementation on cardioplegia COP through an in vitro analysis. A self-contained cardioplegia delivery system administered supplemental ALB to four BCP ratios (1:1, 4:1, 8:1, and 20:1). In Group A, 25% ALB was combined with BCP at four delivery rates (0, 13, 25, and 50 mL ALB/L BCP), with a delivery rate of 0 mL ALB/L BCP serving as the control for all groups. Twenty-five percent ALB was added to crystalloid to create carrier solutions containing 12.5, 25, or 50 g ALB/L in Group B, while Group C combined an ALB delivery rate of 50 mL ALB/L BCP with each of the three carrier solutions. Endpoints included initial and post-supplementation hematocrit, total serum protein (TSP), and COP. Without supplemental ALB, TSP was less affected with increasing blood to crystalloid ratios (1:1-81.7 ± 6.2%, 4:1-40.6 ± 5.1%, 8:1-20.6 ± 4.1%, 20:1-6.0 ± 5.7%). The TSP of 1:1 and 4:1 BCP increased (p < .0003 and p < .02) across all methods of supplementation, while 8:1 BCP was similarly increased (p < .008), except with 12.5 and 25 g ALB/L carrier solutions. The greatest change from baseline COP was seen with the lower blood to crystalloid ratios (1:1-64.3 ± 5.0% and 4:1-39.5 ± 10.5%). In higher ratios, the effects of dilution were less profound (14.6 ± 4.2 ± 4.2% and 20:1-6.0 ± 1.9%). COP of 1:1 BCP increased (p < .008) whenever ALB was added. In conclusion, TSP and COP of blood cardiopugic solutions is increased by supplemental albumin administration with quantitative enhancement dependent upon the dilutional effects of the blood to crystalloid ratio. Keywords: colloid oncotic pressure, albumin, myocardial edema, myocardial protection. JECT. 2003;35:17–23

The elimination of air from the extracorporeal circuit dictates fluid priming before the initiation of cardiopulmonary bypass (CPB). Priming components may be sanguineous, asanguineous, or a combination of the two. Often, the priming solution is asanguineous, consisting of crystalloid solutions (1). Pure crystalloid priming dilutes the patient’s own circulating volume, reducing hematocrit (Hct) and significantly decreasing plasma protein concentration (2). Hct and plasma protein concentrations may be further reduced throughout CPB during cardiopugia administration and, with addition of crystalloids, to maintain adequate reservoir volumes. Various studies have shown reductions in Hct and in arterial oxygen content are not deleterious because various compensating mechanisms can guarantee tissue oxygenation and systemic oxygen transport (3). However, dilution of the plasma protein concentration is accompanied by a substantial reduction in colloid osmotic pressure (COP), which has been linked to compromised pulmonary function and widespread organ tissue edema (3).

Under normal circumstances, COP ranges from 22–28 mmHg, with approximately 78% of this pressure caused by albumin (ALB) in the plasma. Starling’s equation utilizes the primary forces at the capillary membrane as well as the reflection coefficient of the membrane to determine the rate of fluid filtration at the capillary membrane. A
summation of these forces reveals a total outward or extravascular force of 28.3 mmHg and a total inward or intravascular force of 28 mmHg. The excess fluid forced into the interstitium by the net outward force of 0.3 mmHg pressure is usually taken into the lymphatic system and redistributed to the intravascular space (4). However, decreased COP in combination with increased vascular permeability leads to a net flux of fluid from the intravascular space to the extravascular space (2), ultimately causing edema.

Suboptimal COP of cardioplegic solution may lead to myocardial edema, which can impair systolic and diastolic function. Mehlhorn et al. note that the plegic myocardium is especially prone to edema formation because of relatively enhanced fluid filtration as well as lymph flow cessation (5). The development of myocardial edema in neonates is of special concern because it often requires that the sternum be left open, contributing to longer ICU and total hospital stay of such patients (6). One approach to combat falls in COP of cardioplegic solutions is to administer ALB. The purpose of this study was to quantify the effects of ALB supplementation during cardioplegia administration on total serum protein (TSP), and COP of cardioplegic solution.

MATERIALS AND METHODS

Circuit Preparation

The test circuit was modified from a circuit designed by Sydzyik, et al. (7) (Figure 1). The circuit included a reservoir containing 30 L fresh bovine blood, a cardiotomy reservoir (Sorin Biomedica, Arvarda, CO), two roller pumps (Stockert, Sorin Biomedical, Inc., Irvine, CA), and the Myocardial Protection System™ [(MPS), Quest Medical, Inc., Allen, TX]. The MPS was selected because of the ability to change various components of cardioplegic solution independently, which allowed for uncomplicated modification of blood to crystalloid ratios and ALB delivery rates. The system has two components, a microprocessor controlled electromechanical instrument and a disposable delivery set. The disposable set includes valved pouches that alternatively fill and pump blood and crystalloid as well as two separate pouches for the arresting agent and additive agent of choice. A complete device description can be found elsewhere (7). Blood from the reservoir was continuously circulated at 2 L/min, and blood was transferred into the cardiotomy reservoir as needed. Distal to the second roller pump, a cardioplegia heat exchanger (Sorin Biomedica, Arvarda, CO) was inserted and used to maintain a circulating blood temperature of 37 ± 1°C (Dual Heater-Cooler, Terumo Cardiovascular, Ann Arbor, MI). Blood from the cardiotomy reservoir was circulated at 1.25 ± 0.25 L/min. Distal to the heat exchanger a 1/4×1/4×1/4-inch wye connector with Luer lock for sampling was inserted, linking the cardiotomy blood to the MPS. The outlet line of the MPS was modified to include a 3/16×3/16×3/16-inch connector with Luer lock for sampling and additional 3/16-inch polyvinyl chloride (PVC) tubing to direct modified blood into either the cardiotomy reservoir or a waste container.

Solutions and Blood Preparations

All crystalloid sources were elevated 60 cm above the MPS. In group A, the crystalloid source was 1L of physiologic saline solution (PSS); 12.5 g of 25% ALB was added to PSS to create carrier solutions containing 12.5, 25, or 50 g ALB/L for use in groups B and C. The bovine blood source was anticoagulated with heparin (2500 iu/L blood), and anticoagulation level was assessed using an activated clotting time (ACT) machine. ACT times were maintained 480 seconds and were verified every 60 minutes of experimentation. PSS was added to the blood source to achieve a starting Hct of 28 ± 2%.

Experimental Conditions

The MPS was primed and debubbled according to the manufacturer’s directions for use. Cardioplegia delivery temperature was set at 37 ± 1°C. Supplemental ALB was administered to four blood cardioplegia ratios (1:1, 4:1, 8:1, and 20:1). In group A, 25% ALB was combined with BCP at four delivery rates (0, 13, 25, and 50 mL ALB/L BCP), with a delivery rate of 0 mL ALB/L BCP serving as the control for all groups. Delivery rates of 13, 25, and 50 mL ALB/L BCP were designated treatments 1, 2, and 3, respectively. Twenty-five percent ALB was added to PSS to create carrier solutions containing 12.5, 25, or 50 g
ALB/L in group B, while group C combined an ALB delivery rate of 50 mL ALB/L BCP with each of the three carrier solutions. For group B, concentrations of 12.5, 25, and 50 g ALB/L were designated treatments 1, 2, and 3, respectively, and the combined group C treatments were labeled in the same manner.

For group A, all cardioplegia ratios and ALB delivery rates were varied in randomized fashion, and all combinations were repeated in quadruplicate. In groups B and C, where carrier solutions were used, randomization occurred within carrier solution treatments, and all combinations were repeated in duplicate. Between successive sample times, a washout was performed to prevent contamination of measured parameters. This involved setting the device to the next blood cardioplegia ratio and albumin delivery rate and allowing 200 mL of solution to pass through the circuit into a waste container.

**Sampling and Measurement**

Initial and postsupplementation HCT, TSP, and COP were recorded for each experimental combination. One hundred fifty µL of sampled blood was placed in heparinized capillary tubes and sealed with clay. The tubes were spun in a centrifuge for 4 minutes at 10,000 rpm, and HCT of the samples were determined. The plasma was retained for TSP analysis using the American Optical Refractometer [(AOR), American Optical Company, Buffalo, NY]. The AOR was calibrated using distilled water and 0.9% NaCl. All HCT and TSP measurements were performed in at least duplicate. 10 mL of sampled blood was stored at 4°C for 3 days before determining COP with a membrane Colloid Osmometer 4420 (Wescor, Inc., Logan, UT). The osmometer was calibrated with Osmocoll [(TSP of 6.3 g/dL and COP of 20.2 ± 0.5 mmHg) Wescor, Inc., Logan, UT].

**Statistical Analysis**

All data were loaded onto a personal computer in spreadsheet format. Percentage change in HCT, TSP, and COP were analyzed using a two-way analysis of variance (ANOVA) with repeated measures. When significant effects were found, a post hoc test (Fisher’s least significant difference) was performed. All tests were two-sided, and statistical significance was accepted at the $p \leq .05$ level. All data are reported as mean ± standard deviation of the mean.

**RESULTS**

Before experimentation, a standard curve was developed using serial dilutions of 25% ALB in crystalloid. TSP and COP of the resulting solutions were analyzed. From the regression of COP (mmHg) on TSP (g/dL), it was determined that as TSP increased, COP increased exponentially ($R^2 = 0.9695$) (Figure 2). The equation that can be used to estimate COP with TSP follows.

$$\text{COP} = 5.7914e^{0.2779 \times \text{TSP}}$$

where $e = 2.7182$.

The TSP concentrations used to estimate this equation ranged from 1.0 g/dL to 8.0 g/dL, and estimates of COP should not be extrapolated from TSP concentrations beyond this range. It should also be noted that the values are based on measurements taken from a crystalloid “prime” with additive ALB.

The percentage change between initial and postsupplementation HCT, TSP, and COP were determined for all experimental combinations. As expected, percentage change in HCT was significant ($p < .0001$) for all comparisons, except 8:1 vs. 20:1. The mean changes in HCT across all groups were as follows: 1:1, −48.95 ± 1.90%; 4:1, 22.51 ± 3.55%; 8:1, −8.52 ± 1.47%; 20:1, −5.34 ± 2.85%. Change in HCT did not correspond with change in TSP and COP to a greater degree than the change in HCT might suggest. Table 1 shows the percentage change in TSP and COP for all groups and treatments within each BCP ratio.

TSP decreased for all blood to crystalloid ratios when no ALB supplementation was used. The analysis of percentage change in TSP across blood to crystalloid ratios is shown in Figure 3. All methods of supplementation, except group A, treatment 1 significantly ($p < .0003$) increased the TSP of 1:1 BCP. Group B, treatment 1 did not significantly increase the TSP of 4:1 BCP, but all other methods did ($p < .02$). TSP of 8:1 BCP was significantly increased ($p < .008$) except when group A, treatment 1 and group B, treatments 1 and 2 were used. None of the group C treatments significantly increased the TSP of 20:1 BCP; however, all other methods did ($p < .007$). Figure 3 demonstrates that supplementing ALB with carrier solutions was not the most effective way to increase TSP of 8:1 or

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**Figure 2.** Standard curve. Total serum protein effects on colloid osmotic pressure. COP = colloid osmotic pressure; $e = 2.71828$; TSP = total serum protein.
Table 1. Raw data.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>TSP (mL/L)</th>
<th>COP (% Change)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
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<td>A</td>
<td>Baseline</td>
<td>0</td>
<td>-15 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>T.1</td>
<td>21 ± 4</td>
<td>-8 ± 6</td>
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<tr>
<td></td>
<td>T.2</td>
<td>29 ± 5</td>
<td>-4 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>T.3</td>
<td>38 ± 6</td>
<td>1 ± 1</td>
<td>&lt; .01</td>
</tr>
<tr>
<td>B</td>
<td>T.1</td>
<td>12.5</td>
<td>2 ± 4</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>T.2</td>
<td>25</td>
<td>2 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>T.3</td>
<td>50</td>
<td>3 ± 7</td>
<td>NS</td>
</tr>
<tr>
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<tr>
<td></td>
<td>T.3</td>
<td>50 + 50</td>
<td>15 ± 11</td>
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</table>

DISCUSSION

In the pediatric population, capillary permeability is naturally higher (8), and COP may fall as much as 40% on CPB (9). COP falls both because of higher capillary permeability and the high ratio of prime to patient blood volume. Thus, it is not surprising that Maehara et al. designate edema as one of the most obvious deleterious effects of CPB, with or without circulatory arrest, in neo-
nates and young infants (10). The ideal COP during neonatal bypass remains in question (11), although an animal model (infant rabbits) suggests that the optimum COP and total protein (TP) concentration during CPB are 16 mmHg and 4.2 g/dL, respectively (12). A TP concentration of 4.2 g/dL theoretically corresponds to an ALB concentration of about 3.3 g/dL (78% of 4.2).

Retrospective analysis of records of children receiving crystalloid prime compared with those receiving whole blood prime suggests that maintained COP levels during CPB reduce fluid accumulation, and that lower COPs are associated with a longer intensive care unit (ICU) stay and higher mortality (13). Aukerman et al. concluded that adding ALB to the extracorporeal circuit prime along with minimizing prime volume results in a decrease in postoperative weight gain in children undergoing CPB (14). An infant animal study has demonstrated that increasing the HCT and increasing COP with pentastach during the cooling period before deep hypothermic circulatory arrest (DHCA) improves postoperative cerebral recovery (6).

Although the development of peripheral edema in the pediatric patient is a concern, of greater concern is the tendency toward myocardial edema. The prevention of myocardial edema in the pediatric population is important because it impairs systolic and diastolic cardiac function (5) and because the development of edema in neonates often requires that the sternum be left open, contributing to longer ICU and total hospital stay of such patients (6). It has been shown that increasing the COP of normothermic blood cardioplegia (4:1) minimizes myocardial edema, preventing post-CPB cardiac dysfunction (5).

Mehlhorn, et al. note that the plegic myocardium is especially prone to edema formation because of relatively enhanced fluid filtration as well as lymph flow cessation (5). These researchers used 6% hetastarch to achieve a blood cardioplegia COP of 21 ± 2 mmHg at a delivery perfusion pressure of 50 mmHg in an animal model, confirming that colloidal priming can avoid myocardial edema caused by hemodilution induced by CPB. Extrapolation of their data indicated that the COP necessary to achieve a microvascular filtration rate approaching zero and avoid myocardial weight gain would require a COP of around 36 mmHg for normothermic, continuous blood cardioplegia.

The use of ALB in prime to combat the reduction in COP caused by hemodilution on CPB is somewhat con-
For those utilizing ALB for general volume expansion, the product insert for 25% Human Albumin advises that the plasma ALB concentration necessary to prevent or reverse peripheral edema is unknown and undoubtedly varies from patient to patient, but is thought to be near 2.5 g/dL. A goal of maintaining a plasma ALB concentration of about 2.5 ± 0.5 g/dL or a plasma (colloid) oncotic pressure of 20 mmHg, the equivalent of a total protein (TP) concentration of 5.2 g/dL, should be sought. It should be noted that an ALB concentration of less than 3.5 g/dL is regarded as the lower limit of normality by most authorities, with clinical features such as edema appearing at concentrations below 2.0 g/dL (21). Several studies support that low ALB levels, less than 3.5 g/dL in some and less than 2.5 g/dL in others, are linked to increased morbidity and mortality (16,17, 21–25). These studies involved various patient populations, including the generally critically ill, moribund surgery patients, as well as cardiac surgery patients. One study indicates that a post-operative COP of 15 mmHg or less is associated with a 50% survival rate of the critically ill (17). Based on information from these studies, it is advisable not to let ALB levels fall below 3.5 g/dL during CPB. Also, it may be desirable to maintain COP above 15 mmHg intraoperatively.

Current practice usually includes adding ALB to asanguineous primes, and some clinicians add ALB to sanguineous primes as well. However, the amount of ALB added is often empiric and may not adequately maintain ALB concentration, TSP, or COP. The standard curve developed in this study demonstrates that adding 25 g ALB per 1000 mL of sanguineous prime results in a TSP of 1.55 g/dL and a COP of only 8.6 mmHg (Figure 2). The amount of ALB that must be added to asanguineous primes to achieve a TSP of 3.15 g/dL and a COP of around 15 mmHg is higher than might be expected and requires that 42 g ALB be added per 1000 mL of prime. Increasing the prime COP to this level will certainly reduce the dilution of the plasma protein concentration to a minimum; however, the optimum ALB concentration, TSP, and COP in priming solutions and during CPB should be further investigated.

Once an optimal prime ALB concentration, TSP, and COP are delineated or designated by the clinician, the same should be addressed in cardioplegic solutions. TSP and COP decrease for all blood to crystalloid ratios when no method of ALB supplementation is used. In this study, TSP and COP were best maintained without supplementation by 20:1 BCP. The regression equations developed from our results indicate that the most simplistic way to maintain a zero percentage change from baseline COP with 1:1 BCP is to add 30 g ALB/L crystalloid cardioplegia solution. For 4:1 BCP, slightly more ALB must be added, 37 g/L.

Future studies could include repeating the study with whole human blood to verify that the equations developed using bovine blood are valid for human blood as the authors expect they should be. In addition, PSS was used as the crystalloid source in this study, and repeating the study with a traditional cardioplegic solution may be called for. Recreation of the Melhorn et al. study using a neonatal
animal model and the techniques described in this paper may also be necessary. The sample size for a laboratory study should be sufficiently large to achieve statistical significance. After confirming that increasing the COP of hypothermic blood cardioplegia minimizes myocardial edema and prevents post-CPB cardiac dysfunction in the animal model, the techniques could be applied in a clinical study setting.

In conclusion, it is recommended that a minimum ALB concentration of 3.5 g/dL and a minimum COP of 15 mmHg be accepted during CPB. The standard curve developed in this study can be used to adjust prime COP based on TSP measured by refractometer. The regression equations developed for supplementing ALB in 1:1, 4:1, 8:1, and 20:1 BCP (Table 2) can be used to adjust COP of BCP based upon either ALB delivery rates in mL ALB/L BCP or upon g ALB/L crystalloid. In addition, these equations may be used to determine what ALB delivery rate is necessary to maintain a selected or baseline COP. The grams of ALB to add per liter of crystalloid to maintain a predetermined COP may also be calculated with these equations.

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