Comparison of the Albumin, Colloid Osmotic Pressure, and Coagulation Factors in Canine Plasma Products and the Clinical Use of Cryopoor Plasma in Hypoalbuminemic Canine Patients

THESIS

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

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2016

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Abstract

Objective— To determine albumin and coagulation factors levels and colloid osmotic pressure (COP) of cryoprecipitate (CRYO), and cryopoor plasma (CPP), compared to the source fresh frozen plasma (FFP). To evaluate the potential impact of CPP continuous rate infusion (CRI) on albumin and COP in critically ill canine patients with documented hypoalbuminemia.

Setting— University Veterinary Teaching Hospital Blood Bank and Intensive Care Unit.

Interventions— Source FFP was separated into CRYO and CPP and the COP, albumin, von Willebrand factor (vWF), fibrinogen and coagulation factors II, V, VII, VIII, IX, and X were assessed for each product. A retrospective study of 10 hypoalbuminemic dogs receiving a CPP infusion for albumin replacement was performed to evaluate the potential impact of CPP on albumin and COP.

Measurements and Main Results— The fibrinogen levels of CRYO (median 379.5 mg/dL, 95% CI 279-491.03), factor VIII (mean 427.0%, ±95.4) and vWF (mean 504.7%, ± 41.39) were significantly increased compared to the other products. The mean albumin and COP in CPP were significantly higher than levels in FFP, with 3.17 g/dL (±0.6) in CPP compared to 2.89 g/dL (±0.05) in FFP (p = < 0.001) and 14.5 mmHg (±0.65) in CPP compared 12.73 mmHg (± 0.31) in FFP (p = 0.03). The levels of vitamin K dependent factors II, VII, and X were similar in CPP and FFP and factor IX was highest in CRYO.
In the clinical portion, 7/10 dogs were septic, with 29% of those being in septic shock and 71% having septic peritonitis. The mean pre- and post-infusion albumin were 15.4 g/L (±3.9) and 21 g/L (±2.5), respectively (p = 0.011). The median pre- and mean post-infusion COP were 8.6 mmHg (4.9-9.7) and 10.5 mmHg (±1.5), respectively (p = 0.0039). The mean rate of CPP administered was 1.8 mL/kg/hr (±0.6), the mean rate of crystalloid administered was 0.8 mL/kg/hr (±0.9), and the mean rate of HES administered was 1.2 mL/kg/hr (±0.9). Delta albumin was positively correlated with CPP mL/kg/hr (p = 0.0004), while delta COP was positively correlated with HES mL/kg/hr (p = 0.0128). No side effects of CPP infusion were reported. Age was the only factor associated with survival (p = 0.033) with non-survivors more likely to be older. 30% of patients died, 30% of patients were euthanized, and 40% of patients survived to discharge. All discharged patients survived to 90 days post-discharge.

**Conclusions**— CRYO contained a high concentration of vWF and factor VIII. CPP had the highest levels of albumin and COP and the levels of factors II, VII, and X were similar to FFP. The in vitro study found that the dose of CPP administered was correlated with an increase in albumin, but not with an increase in COP. CPP may be a valid option for treatment of hypoalbuminemia and low COP, however further clinical studies are warranted regarding dose, safety and impact on outcome.
Dedication

Dedicated to my father, who gently asked me to finish my residency, not realizing what that would mean.
Acknowledgements

I would like to thank Cristina Izabik, DVM and Jessica Fussnecker, RVT for all of their help in the blood bank collecting samples and processing blood. To Jana Fletcher, RVT for her help in the processing of samples.

Thank you to all of the doctors and staff on the Emergency and Critical Care service who helped care for the patients in this study. To Dr. Julien Guillaumin, my advisor, for the inspiration for this study.

To Scott Stilwell, for his understanding and endless support at home.

Thank you to my mother, my sounding board.

I would also like to thank the Veterinary Emergency and Critical Care Society Foundation for the grant that provided funding for this project.
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Major Field: Comparative and Veterinary Medicine
Small Animal Emergency and Critical Care Residency
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Chapter 1: Introduction

1.1 Albumin and Colloid Osmotic Pressure

Albumin is a protein that is 66 kDa in size, containing 584 known amino acids (Figure 1.1).\textsuperscript{1} It is synthesized in the liver at a rapid rate, around 12-25 grams per day, however excessive albumin loss through conditions such as loss through the intestines, kidneys, or effusion can overwhelm the liver’s ability to synthesize sufficient amounts of albumin.\textsuperscript{1,2} The synthesis is regulated by hepatic osmoreceptors, however with artificially increased colloid osmotic pressure (COP), such as with administration of exogenous albumin or synthetic colloids, the trigger for synthesis will be disturbed and synthesis will be decreased.\textsuperscript{1} Approximately 10% of albumin is metabolized daily. In dogs, the half life is 8.2 days, with the half life being inversely related to concentration.\textsuperscript{1,3}

![Figure 1.1. Crystal structure of human serum albumin. From RCSB Protein Data Bank.](image)

Approximately 30-40% of albumin is present in the intravascular space, with the remaining 60-70% being extravascular.\textsuperscript{1} Transcapillary shifting of albumin is normally around 5% of the
intravascular content hourly and increases with various conditions including infection, sepsis, shock, burns, and catecholamines.\textsuperscript{1} Albumin is responsible for 70-80\% of the colloid osmotic pressure (COP), which helps to decrease loss of plasma from the capillaries.\textsuperscript{1,2} The oncotic contribution of albumin is important in the fluid shifting at the level of the capillaries. Part of this effect comes from the attraction of sodium for albumin, which further enhances water retention and resorption within the vasculature, an effect known as the Gibbs-Donnan effect. As seen in the net filtration pressure equation below, first describe by Ernest Starling, the plasma oncotic pressure plays a large role in the net filtration pressure.

\[
\text{Net filtration pressure} = K_f(P_c - P_i) - \sigma(\Pi_p - \Pi_i)
\]

\(K_f = \text{filtration coefficient}\)
\(P_c = \text{capillary hydrostatic pressure}\)
\(P_i = \text{interstitial hydrostatic pressure}\)
\(\Pi_p = \text{plasma oncotic pressure}\)
\(\Pi_i = \text{interstitial oncotic pressure}\)
\(\sigma = \text{reflection coefficient}\)

Albumin serves a variety of functions, including drug binding, hormone and drug transport, anti-apoptotic effects, beneficial effects on vascular integrity, and acid-base buffering.\textsuperscript{4} It can bind toxic substances and can act as a free radical scavenger, decreasing production of reactive oxygen species through binding of iron and copper, and directly scavenging reactive oxygen species.\textsuperscript{4} Albumin can have anti-coagulation and anti-thrombotic properties through inhibition of platelet function and heparin-like effects such as anti-Xa activity.\textsuperscript{4}

Hypoalbuminemia can occur from a variety of causes, such as hemorrhage, third-spacing, and renal or intestinal losses.\textsuperscript{5-9} Due to its influence on oncotic pressure, hypoalbuminemia can be associated with formation of edema and third spacing of fluids due to decreased oncotic
pressures.\textsuperscript{2} This has the potential to cause profound fluid loss and hypotension secondary to hypovolemia. Attempts to restore the vascular volume with isotonic crystalloids may exacerbate interstitial edema and third spacing, with only a transiently positive effect on intravascular volume and blood pressure due to fluid shifting. Thirty minutes after administration, less than one third of the volume of isotonic crystalloid administered remains in the intravascular space due to fluid redistribution according to Starling’s equation.\textsuperscript{10} In hypoalbuminemic patients, a colloidal solution is recommended, as it will help to improve the oncotic status of the patient and decrease fluid losses from the capillaries. Preoperative hypoalbuminemia in canine and feline patients undergoing gastrointestinal surgery has been found to be associated with a 3.6 times higher relative risk of mortality, 6.4 times higher risk of complications, and a need for longer hospital stay.\textsuperscript{11} In humans, each 10 g/L decrease in albumin increases odds of mortality by 137\%, morbidity by 89\%, and prolongs hospitalization in the Intensive Care Unit (ICU) by 28\%.\textsuperscript{12} In septic human patients, albumin replacement has been shown to be beneficial.\textsuperscript{13,14} The SAFE study compared the use of a 4\% albumin solution to normal saline for fluid resuscitation in human ICU patients.\textsuperscript{14} The septic subgroup was found to have increased survival in the patients administered 4\% albumin as compared the patients treated with normal saline. The ALBIOS study also found improved survival in human septic shock patients treated with 20\% albumin and crystalloids as compared to patients treated with crystalloids alone.\textsuperscript{13}

Colloidal solution fall into two categories, natural and synthetic, each of which are associated with potential adverse effects. The estimated colloid osmotic pressure (i.e. oncotic pressure) of these products is highlighted in table 1.1. Synthetic colloids include hydroxyethyl starches (HES) such as HES 6\% 130/0.4/9:1, a product that is appealing because it is relatively inexpensive, easy to store, requires no preparation or special infusion equipment, and is readily available in the
<table>
<thead>
<tr>
<th>Fluid</th>
<th>COP (mmHg)</th>
<th>Osmolarity (mOsm/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6% hetastarch</td>
<td>32.7 ± 0.2</td>
<td>308</td>
</tr>
<tr>
<td>6% dextran 70</td>
<td>61.7 ± 0.5</td>
<td>309</td>
</tr>
<tr>
<td>Canine FFP</td>
<td>17.1 ± 0.6</td>
<td>N/A</td>
</tr>
<tr>
<td>25% human albumin</td>
<td>&gt; 200</td>
<td>294</td>
</tr>
<tr>
<td>12.5% human albumin</td>
<td>95.3 ± 0.5</td>
<td>302</td>
</tr>
<tr>
<td>5% human albumin</td>
<td>23.2 ± 0.1</td>
<td>306</td>
</tr>
<tr>
<td>Lactated Ringers</td>
<td>0.0 ± 0.0</td>
<td>273</td>
</tr>
<tr>
<td>Plasmalyte A</td>
<td>0.0 ± 0.0</td>
<td>294</td>
</tr>
<tr>
<td>Hypertonic saline</td>
<td>0.4 ± 0.1</td>
<td>2464</td>
</tr>
<tr>
<td>25% mannitol</td>
<td>1.3 ± 0.2</td>
<td>1372</td>
</tr>
</tbody>
</table>

Table 1.1. Colloid osmotic pressure (COP) and osmolarity measurements of various fluids using a membrane with 90% rejection rate at 30 kD (mean ± SD). FFP = fresh frozen plasma. Adapted from Chan et al.18

United States. However, in humans, HES are known to have significant side effects such as acute kidney injury in some patient populations, which may further compromise an already critical patient.15,16 The Surviving Sepsis Campaign Guidelines recommend against the use of hydroxyethyl starches and suggest using albumin instead in cases of severe sepsis and septic shock.15 Additionally, large volumes of hydroxyethyl starches have been showed to potentially cause coagulopathies, which could also be detrimental to patients.17 Synthetic colloids also do not contain albumin and as such do not provide the additional benefits of albumin containing products, such as drug transport.

Natural colloids include plasma products such as FFP, CPP, and albumin concentrates. 25% human serum albumin (HSA) is sometimes utilized in critical canine patients to increase albumin. It contains a higher albumin concentration than plasma, however there are legitimate concerns about type I and III hypersensitivity reactions based on canine studies and no prospective studies have yet been published on its use in critical canine patients.19,20 As such, it is typically reserved for critically ill patients and repeated transfusions in a single patient are prohibited.19,21 Canine-
specific albumin replacement would be the ideal option. Unfortunately no canine albumin concentrate is currently available, so species-specific albumin replacement for canine patients is limited to less concentrated products such as FFP, CPP, or 5% lyophilized canine albumin. There is one study investigating the change in albumin levels following administration of 5% lyophilized canine albumin solution. An increase in albumin levels was found in dogs with septic peritonitis that received that product and one dog developed tachypnea during the albumin transfusion. This 5% lyophilized canine albumin is commercially available, however its availability has been inconsistent. FFP transfusion is safe, as transfusion reactions are infrequent, occurring in 1-3% of patients, and reactions are most often mild such as fever and pruritus. A previously published study evaluating the content of FFP reported albumin 20-30 g/L and COP 17-20 mmHg, with no difference between Greyhounds and non-Greyhounds. This shows promising results for albumin content, however it represents a ~3% albumin solution as compared to 25% albumin in HSA.

In veterinary medicine, the equation to calculate the albumin deficit is usually cited:

\[
\text{Albumin deficit (g)} = [(\text{desired albumin}) - (\text{patient albumin})] \times \text{body weight} \times 0.3
\]

where albumin is reported in g/dL and body weight reported in kg.

Using this equation, 10 mL/kg would be needed to increase the albumin by 1 g/dL. However, the origin of this equation is uncertain and the amount of albumin that should be administered is still controversial, with human studies using a variety of doses. Given the dynamic nature of a critically ill patient, simply basing the albumin dose on simply a calculated deficit may not be sufficient to achieve a desired albumin level, hence why most human studies administered
albumin at a fixed daily dose between 0.4 and 0.9 g/kg/day and titrated albumin administration to
a target albumin level.\textsuperscript{13,14}

Finding a safe, effective, and cost efficient source of albumin for canine patients would
potentially benefit critically ill, hypoalbuminemic patients and more research in this area is
warranted.

1.2 Coagulation Factors

The various coagulation factors are listed in Table 1.2. These factors are involved in the
formation of a clot through a complex cascade of chemical reactions outlined below.

\textbf{Table 1.2.} Coagulation factors and their synonyms. Adapted from Hall and Guyton.\textsuperscript{2}

<table>
<thead>
<tr>
<th>Coagulation Factor</th>
<th>Alternate Name</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor I</td>
<td>Fibrinogen</td>
<td></td>
</tr>
<tr>
<td>Factor II</td>
<td>Prothrombin</td>
<td>Vitamin K dependent</td>
</tr>
<tr>
<td>Factor III</td>
<td>Tissue factor</td>
<td></td>
</tr>
<tr>
<td>Factor IV</td>
<td>Calcium</td>
<td></td>
</tr>
<tr>
<td>Factor V</td>
<td>Proaccelerin, Ac-globulin</td>
<td></td>
</tr>
<tr>
<td>Factor VII</td>
<td>Proconvertin, serum prothrombin conversion accelerator</td>
<td>Vitamin K dependent</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Antihemolytic factor, antihemophilic globulin, antihemophilic factor A</td>
<td>Deficit = Hemophilia A</td>
</tr>
<tr>
<td>Factor IX</td>
<td>Plasma thromboplastin component, Christmas factor, antihemophilic factor B</td>
<td>Vitamin K dependent</td>
</tr>
<tr>
<td>Factor X</td>
<td>Stuart factor, Stuart-Power factor</td>
<td>Vitamin K dependent</td>
</tr>
<tr>
<td>Factor XI</td>
<td>Plasma thromboplastin antecedent, antihemophilic factor C</td>
<td></td>
</tr>
<tr>
<td>Factor XII</td>
<td>Hageman factor</td>
<td></td>
</tr>
<tr>
<td>Factor XIII</td>
<td>Fibrin-stabilizing factor</td>
<td></td>
</tr>
</tbody>
</table>
Two approaches to hemostasis have been discussed in medicine, the coagulation cascade and the cell-based model to coagulation. The cascade allows a simplistic, stepwise approach to coagulation as is outlined in Figures 1.3-1.5, however it best reflects in vitro situations and does not incorporate the effects of the endothelium on coagulation. It is however useful for explaining the clotting times used on the clinical floor. The cell-based model more accurately reflects coagulation in vivo and is detailed in Figure 1.2.

**Figure 1.2.** The cell-based model of fibrin formation. The cell-based model incorporates the contribution of various cell surfaces to fibrin formation. In this model thrombin generation occurs in overlapping phases. (a) Initiation Phase. This phase occurs on the TF-bearing cell. It is initiated when injury exposes the TF-bearing cell to the flowing blood. It results in the generation of a small amount of FIXa and thrombin that diffuse away from the surface of the TF-bearing cell to the platelet. (b) Amplification Phase. In the second phase, the small amount of thrombin generated on the TF-bearing cell activates platelets, releases vWF and leads to generation of activated forms of FV, FVIII, and FXI. (c) Propagation Phase. In the third phase the various enzymes generated in earlier phases assemble on the procoagulant membrane surface of the activated platelet to form intrinsic tenase, resulting in FXa generation on the platelet surface. Prothrombinase complex forms and results in a burst of thrombin generation directly on the platelet. Figure and accompanying text from Smith 2009.27
**Factor I – Fibrinogen**

Fibrinogen has a molecular weight of 340,000 and is formed in the liver. As such, hepatic disease can potentially lead to a decrease in fibrinogen. Fibrinogen is an essential factor for coagulation. Because fibrinogen is large, it normally does not readily leak into interstitial fluid from the blood, decreasing the potential of coagulation within the interstitium.

![Diagram of coagulation process](image)

**Figure 1.3.** Simplified version of coagulation. From Guyton 2016.

**Factor II – Prothrombin**

Prothrombin is an α2-globulin with a molecular weight of 68,700. It is also formed constantly in the liver, with the potential for bleeding if the liver fails to produce prothrombin. It is activated by vitamin K, as discussed below. Prothrombin is converted to thrombin in the presence of calcium and prothrombin activator.
**Factor III – Tissue Factor**

Factor III is composed of phospholipids and a lipoprotein complex and is released when there is damage to the vascular wall. It then forms a complex with factor VII and activates factor X (Figure 1.4).

![Diagram of the extrinsic pathway for coagulation](image)

**Figure 1.4.** Extrinsic pathway for coagulation. Ca^{++} = calcium. From Guyton 2016.

**Factor V – Proaccelerin, Ac-globulin**

Factor V is complexed with phospholipids and activated factor X as prothrombin activator. As thrombin forms, its proteolytic activity results in activation of factor V. Activated factor V accelerates prothrombin activation.
**Figure 1.5.** Intrinsic pathway for coagulation. $\text{Ca}^{++} = \text{calcium}$. From Guyton 2016.$^2$

*Factor VII – Proconvertin, serum prothrombin conversion accelerator*

Activated factor VII complexes with tissue factor to activate factors IX and X (Figure 1.4).

*Factor VIII – Antihemolytic factor, antihemophilic globulin, antihemophilic factor A*

Activated factor VIII, along with activated factor IX, factor III, and platelet phospholipids cause activation of factor X (Figure 1.5).$^2$ A deficiency of factor VIII, hemophilia A, results in deficiency of this step in coagulation.$^2$
Factor IX – *Plasma thromboplastin component, Christmas factor, antihemophilic factor B*

Factor IX is activated by activated factor XI. Activated factor IX, activated factor VIII, factor III, and platelet phospholipids cause activation of factor X (Figure 1.5).²

Factor X – *Stuart factor*

Factor X is activated by factor III, which then combines with factor V and phospholipids from tissues or platelets to form prothrombin activator.² This, in combination with calcium, divides prothrombin into thrombin, as explained above (Figures 1.3, 1.5).²

Factor XI – *Plasma thromboplastin antecedent, antihemophilic factor C*

Factor XI is activated by activated factor XII and serves to activate factor IX (Figure 1.5).²

Factor XII – *Hageman factor*

Exposure of blood to the collagen of the vessel wall causes activation of factor XII, converting it into a proteolytic enzyme, which in turn activates factor XI (Figure 1.5).²

Factor XIII – *Fibrin-stabilizing factor*

Factor XIII is activated by thrombin, with calcium as a cofactor. It serves to crosslink the fibrin mesh to help further stabilize the clot.

Coagulation Factor Deficiencies

Vitamin K dependent factors include factors II, VIII, IX, and X. Vitamin K is required for hepatic carboxylase to add a carboxyl group to glutamic acid residues on the immature form of the previously listed factors.² Vitamin K antagonist rodenticides create coagulopathies through
vitamin K antagonism, resulting in life-threatening hemorrhage due to lack of activated vitamin K dependent coagulation factors. Treatment of this condition includes long-term administration of vitamin K, as well as plasma transfusions to replenish vitamin K dependent factors in patients with active hemorrhage. A similar phenomenon may also be seen with biliary stasis secondary to biliary obstruction or hepatic disease, as the secretion of bile into the gastrointestinal tract is needed for absorption of vitamin K through the digestive tract.\(^2\)

Congenital deficiencies of various coagulation factors are also seen in veterinary medicine. Hemophilia A, a deficiency of factor VIII, is seen in breeds including German shepherd dogs and Labrador retrievers and may be treated with CRYO administration.\(^{28-30}\) Hemophilia B, a deficiency of factor IX, is reported in numerous breeds, including the Deutsch Drahthaar.\(^{29}\) Deficiency of vWF is most commonly observed in Doberman Pinchers and may be treated with administration of desmopressin acetate (DDAVP) to stimulate a transient release of pre-formed vWF from endothelial cells and macrophages.\(^{31}\) An alternative is administration of CRYO, which contains a similar amount of vWF as compared to FFP.\(^{30}\)

### 1.2 Canine Plasma Products

A whole blood donation is commonly divided into packed red blood cells and fresh frozen plasma, to allow maximal use of a single donation, depending on the needs of the patient. Fresh frozen plasma contains all coagulation factors and albumin and therefore could be used for the replacement of any coagulation factor, vWF, or albumin. A more tailored approach, however, can be taken by using specific plasma byproducts. FFP can be partially thawed in order to separate
out factors based on their different freezing points, which should in theory separate fibrinogen, factor VIII and vWf in CRYO, leaving the remaining coagulation factors in higher concentrations in CPP (Figure 1.6, Table 1.3). 

<table>
<thead>
<tr>
<th>Plasma Product</th>
<th>Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh frozen plasma</td>
<td>• All coagulation factors</td>
</tr>
<tr>
<td></td>
<td>• vWf</td>
</tr>
<tr>
<td></td>
<td>• Albumin</td>
</tr>
<tr>
<td>Frozen plasma</td>
<td>• All coagulation factors excluding V, VIII</td>
</tr>
<tr>
<td>Plasma frozen &gt; 6 hours after processing or</td>
<td>• vWf</td>
</tr>
<tr>
<td>FFP frozen &gt; 1 year of storage</td>
<td>• Albumin</td>
</tr>
<tr>
<td>Cryoprecipitate</td>
<td>• Fibrinogen</td>
</tr>
<tr>
<td></td>
<td>• Factors VIII, XIII, vWf</td>
</tr>
<tr>
<td>Cryopoor plasma</td>
<td>Presumably:</td>
</tr>
<tr>
<td></td>
<td>• All coagulation factors excluding fibrinogen, VIII, XIII, vWf</td>
</tr>
<tr>
<td></td>
<td>• Albumin</td>
</tr>
</tbody>
</table>

Table 1.3. Various plasma products and their contents. vWf = von Willebrand factor

Chapter 2 discusses in more detail the steps for processing of CPP and CRYO (page 20), however they are superficially outlined in Figure 1.6. An approximately 450 mL whole blood donation will be centrifuged to produce approximately 250 mL of FFP, which is further subdivided into around 50 mL of CRYO and 200 mL of CPP.

**Cryoprecipitate**

There is limited data on the use of CRYO in veterinary medicine. One study found that greater increases in vWF were achieved with CRYO than FFP in dogs with von Willebrand’s disease and found similar increases in Factor VIII with infusions of FFP and CRYO in dogs with hemophilia A. Another study documented that Doberman Pinschers with von Willebrand’s disease that
Figure 1.6. Production of various plasma products from a unit of whole blood.

were treated with CRYO had shortened mean buccal mucosal bleeding time and increases in vWF, however those treated with FFP had an increase in vWF levels but no shortening of buccal mucosal bleeding time.\textsuperscript{33} Neither of these studies, however, investigated levels of vWF and factor VIII in the actual blood products, however the findings do support that CRYO has a high concentration of vWF and factor VIII.

In humans, CRYO is recommended for fibrinogen replacement.\textsuperscript{34} A recent human clinical trial evaluated the feasibility of administration of CRYO shortly after presentation to patients with significant hemorrhage secondary to trauma with the goal of providing early fibrinogen support.\textsuperscript{35} This study found no 28-day mortality difference and that further investigation on outcome is warranted. Another retrospective study found that CRYO was uncommonly administered (3.6\% of patients) to human trauma patients and reported that no mortality difference was observed in patients with hypofibrinogenemia who were treated with CRYO versus those who did not receive CRYO.\textsuperscript{36} In contrast, the MATTERs II Study found that CRYO may independently improve the survival benefit of tranexamic acid in trauma patients requiring red blood cell transfusions.\textsuperscript{37}
A potential benefit of CRYO over FFP is that it provides a high concentration of vWF, fibrinogen, and factor VIII in approximately one fifth the volume. This could be beneficial in patients that are at risk for fluid overload. Additionally, the low volume allows faster administration of an equivalent amount of the above factors.

**Cryopoor Plasma**

There is no previously published data on CPP in veterinary medicine. In humans, CPP is reported to have reduced levels of vWF, fibrinogen, and factor VIII. Its use in human medicine is reported to be primarily for treatment of thrombotic thrombocytopenia purpura (TPP) or in lieu of FFP for plasma exchange. CPP contains high levels of ADAMTS13, a disintegrin and metalloproteinase thrombospondin type 1 motif, also known as vWF cleaving protease, a deficiency of which causes TPP. One study found that CPP may be superior to FFP for treatment of this condition. CPP has been also reported for use in lieu of FFP for the treatment of disseminated intravascular coagulation associated with pancreonecrosis and generalized peritonitis in humans. In veterinary medicine, CPP is listed for use in treatment of vitamin K antagonist rodenticide toxicity and hypoalbuminemia, although there is no published data to support its use for this condition. To the authors’ knowledge, there are not published data regarding the clinical use of CPP in canine patients.

### 1.4 Study Rationale

Canine blood banks, such as the one at The Ohio State University Veterinary Medical Center, face challenges of limited blood products due to the difficulty of obtaining an adequate number of
blood donors to meet their demands. Currently, CRYO is regarded as a more desirable blood product, with CPP being an underutilized by-product. The OSU blood bank has a significant amount of CPP in storage, but this product was rarely used prior to this study, likely because there was limited information on its content or how it may be utilized. Additionally, CPP was frequently discarded because it was not used before its 6-year expiry date. Investigating the content of and uses for CPP could increase revenue from a single blood donation and could avoid discarding this potentially useful and lucrative product. Exploring potential uses for various plasma fractionation products may help increase the number of patients that may benefit from a single donation and would improve blood product stewardship.

As discussed above, selecting the most specific and appropriate product to treat a particular condition is important, not only to maximize the use of a single blood donation, but also to ensure that a patient is administered only what they need and are not potentially at risk for complications from receiving unnecessary blood components. For example, a patient with immune mediated hemolytic anemia has a deficiency of red blood cells, therefore a packed red blood cell transfusion would be best in order to avoid giving the unnecessary volume of plasma contained in whole blood that could increase the chance of fluid overload. If a patient has vWF deficiency, CRYO offers sufficient vWF in approximately 50 mL of volume, rather than an equivalent amount of vWF in approximately 250 mL of FFP.

Cost of treatment is a significant concern in veterinary medicine. Investigating treatment options that produce similar outcomes but at a lesser expense would be very beneficial to veterinary medicine. Because CPP is considered a byproduct of making CRYO, is it often underutilized or even discarded. As a result, the price of a milliliter (mL) of CPP can be half of one mL of FFP,
as FFP is \(~\$1.00/\text{mL}\) and CPP is \(~\$0.50/\text{mL}\). If CPP contains equivalent albumin and vitamin K dependent coagulation factors as FFP, its use could literally cut the cost of canine albumin replacement or treatment of vitamin K antagonist rodenticide toxicity in half. Having data to support the use of CPP would be beneficial to blood banks from a sales perspective, as well as to the clients who may be more readily able to afford a less expensive treatment option.

CPP, however, is not a concentrated albumin product and therefore its use will not overcome the issue of potential volume overload. An additional goal with this study was to evaluate the potential of CPP as a source for manufacturing a concentrated albumin product through fractionation. At this time, fractionated canine albumin is not readily available, but having a concentrated canine albumin product would be extremely beneficial in the field of veterinary medicine. As CPP contains a comparable amount of albumin to FFP, it may be a source for fractionation. Use of CPP in this manner would result in a less expensive albumin concentrate, as the albumin source would be less expensive. If a cost effective canine albumin concentrate were to be available, this would eliminate many of the challenges seen with current options for albumin replacement, such as hypersensitivity reactions to HSA or volume overload and expense associated with FFP transfusions.

In addition to evaluating the content of coagulation factors, vWF, COP, and albumin of parent FFP and its products, CRYO and CPP, this study also investigated the effect of storage on these factors. Although other elements must be considered before transfusing older, stored products, if the measured variables are found to be relatively static or, alternatively, significantly degraded over time, this could provide useful information on the potential efficacy of using long-term stored products for treatment of coagulopathies, hypoalbuminemia, and low COP.
The aim of the benchtop portion of the project was to measure the COP and to determine the concentration of albumin, fibrinogen, factors II, V, VII, VIII, IX, and X, and vWF of CRYO and CPP, as compared to its source plasma. These factors were evaluated at baseline and one year to also evaluate the effect of storage on these factors. This study serves to compare the content of CRYO and CPP to the source FFP to thoroughly investigate how the coagulation factors are divided during the separation process, rather than comparing to past reported reference ranges for FFP. To the authors’ knowledge, there is not published data comparing these values between these products in veterinary medicine. By obtaining this information, a better understanding can be gained as to which plasma products may have clinically useful amounts of albumin and the potential to provide oncotic support.

A retrospective, clinical branch of this project was included in order to investigate the change in COP and albumin in critically ill dogs that received CPP as a continuous rate infusion in the Intensive Care Unit. The goal with this portion of the study was to evaluate the impact CPP may have in the clinical setting on COP and albumin. Establishing the albumin content in CPP was important, however ultimately the value of that information lies in the use of that product in a critically ill patient. Although this portion of the study only evaluated a small number of patients, to the authors’ knowledge it is the first study to report the use of CPP in veterinary medicine.
Chapter 2: Comparison of Albumin, Colloid Osmotic Pressure, von Willebrand Factor, and Coagulation Factors in Canine Cryopoor Plasma, Cryoprecipitate, and Fresh Frozen Plasma

2.1 Abstract

Objective— To determine albumin and coagulation factors levels and colloid osmotic pressure (COP) of cryoprecipitate (CRYO), and cryopoor plasma (CPP), compared to the source fresh frozen plasma (FFP).

Design— In-vitro study.

Setting— University Medical Teaching Hospital Blood Bank.

Animals— Ten healthy, non-Greyhound dogs enrolled in an academic teaching hospital blood donor program.

Interventions— Fresh blood was obtained from canine blood donors and was separated into FFP and packed red blood cells. The source FFP was further separated into CRYO and CPP. COP, albumin, von Willebrand factor (vWF), fibrinogen and coagulation factors II, V, VII, VIII, IX, and X were assessed for each FFP, CRYO, and CPP.

Measurements and Main Results— The fibrinogen levels of CRYO (median 379.5 mg/dL, 95% CI 279-491.03), factor VIII (mean 427.0%, ± 95.4) and vWF (mean 504.7%, ± 41.39) were significantly increased compared to the other products. The mean albumin and COP in CPP were
significantly higher than levels in FFP, with 3.17 g/dL (±0.6) in CPP compared to 2.89 g/dL (±0.05) in FFP (p = < 0.001) and 14.5 mmHg (±0.65) in CPP compared 12.73 mmHg (± 0.31) in FFP (p = 0.03). The levels of vitamin K dependent factors II, VII, and X were similar in CPP compared to FFP and factor IX was higher in CRYO compared to both FFP and CPP. There was no significant difference in factor II or VII levels between the three products.

**Conclusions**— CRYO contained a high concentration of vWF and factor VIII and could be used to treat vWF deficiency and hemophilia A. The mean albumin and COP were highest in CPP, suggesting that CPP may be a potential alternative to FFP for osmotic support and albumin replacement. As vitamin K dependent coagulation factors II, VII and X in CPP were similar to FFP, CPP may be an option for replacement of most vitamin K dependent factors.

### 2.2 Introduction

Blood banks commonly separate whole blood donations into packed red blood cells and fresh frozen plasma (FFP) in order to maximize the use of each donation. Further division of FFP into cryoprecipitate (CRYO) and cryopoor plasma (CPP) can be made. CRYO is made by partially thawing FFP and removing the supernatant, which leaves the precipitate CRYO, theoretically containing factor VIII, fibrinogen and von Willebrand factor (vWF). The supernatant is CPP, also known as cryosupernatant or cryodepleted plasma, presumably containing the remaining contents of FFP. CPP is often considered a useless byproduct from the production of CRYO and is not readily available through commercial veterinary blood banks. Although CPP is sometimes listed for use in vitamin K antagonist rodenticide toxicities, hypoalbuminemia, and immunoglobulin administration, to the authors’ knowledge, there is no published data on the
content of CPP in veterinary medicine.\textsuperscript{44} When available, CPP is approximately half the cost of FFP, therefore evaluation of this product for its potential uses may reveal a more cost effective alternative to FFP for certain conditions and may encourage better blood product stewardship.

The objective of this study was to measure colloid osmotic pressure (COP) and to determine albumin, vWF, and coagulation factors levels of CRYO and CPP as compared to parent fresh frozen plasma. A second objective was to evaluate the stability of the measured variables after 1 year of routine storage. We hypothesized that CRYO would have a higher level of fibrinogen (factor I), vWF and factor VIII than its source FFP and that CPP would be deficient in these factors. Additionally, we hypothesized that CPP would have COP and levels of albumin and vitamin K dependent factors that were similar to its source FFP. The stability of all measured variables, excepting labile factors V and VIII, were hypothesized to be stable after 1 year of routine storage.

2.3 Materials and Methods

Animals

Ten non-Greyhound dogs that were part of a community-based blood donor program were used in this study. Each dog was deemed healthy based on physical exam performed by a veterinarian and normal baseline bloodwork (complete blood count, chemistry profile, \textit{Dirofilaria immitis} antigen, \textit{Anaplasma phagocytophilum} antibody, \textit{Anaplasma platys} antibody, \textit{Borrelia burgdorferi} antibody, \textit{Ehrlichia canis} antibody and \textit{Ehrlichia ewingii} antibody). All dogs were required to be $\geq 1$ year of age, $\geq 22.7$ kg, current on vaccines, and be on no medications that affect coagulation.
This study was approved by the Ohio State University Institutional Animal Care and Use Committee.

**Blood Collection and Processing**

Whole blood from 10 healthy blood donor dogs was collected according to standard operating procedures of the Ohio State University Veterinary Medical Center Blood Donor Program. For donation, the dogs were placed in lateral recumbency without sedation and the jugular vein aseptically prepared. A 16 g needle that was part of a quadruple collection set was inserted into the jugular vein. Approximately 450 mL of whole blood was collected from each dog. The collection sets of whole blood were centrifuged for 15 minutes at 4657 G (4000 rpm) to separate the plasma from the packed red blood cells (Figure 2.1).

For the purpose of the study, the fresh plasma was transferred into 3 plasma bags containing volumes of 9-30 mL of fresh plasma using a sterile tubing welder. The transfusion lines were steriley removed from these bags and transferred into 4 Eppendorf tubes each containing 1-2 mL of fresh plasma for evaluation of the source plasma content. These aliquots and the remaining fresh plasma (~ 200 mL) was then frozen at -30°C to make FFP.

Within 2 days of being frozen at -30°C, the FFP was placed in a refrigerator at 2-5°C to thaw for 15-20 hours, until it reached a semi-liquid (“slushy”) consistency. The FFP was then centrifuged at 1-6°C at 4657 G (4000 rpm) for five minutes. The precipitate obtained from this process was CRYO while the supernatant was CPP. As per standard protocol, the liquid CPP was then transferred into a satellite bag, with the remaining supernatant being CRYO.[45]
The CPP was equally divided into 3 plasma bags containing 36-80 mL. Four aliquots of 1-2 mL of CPP were obtained from the lines and transferred to Eppendorf tubes using the same procedure as with FFP. The CRYO was similarly processed, with satellite bag volumes of 9-30 mL. One aliquot each of CRYO and CPP were submitted for same day testing of albumin, COP and fibrinogen. All remaining samples were then frozen at -30°C until testing.

Figure 2.1. Stages of processing whole blood into the studied products and the corresponding testing.
CPP = cryopoor plasma; COP = colloid osmotic pressure; vWF = von Willebrand factor

For the objective of assessing variables stability over time, one year after routine storage at -30°C, the aforementioned aliquots of each product were removed from the freezer, allowed to
thaw at room temperature, and thoroughly mixed prior to sampling. All tests were performed on the stored samples (Figure 2.3).

![Diagram](image)

**Figure 2.2.** Aliquot distribution at each time point. Please note that Cornell University requested that two 1 mL aliquots be sent for testing.

![Diagram](image)

**Figure 2.3.** Stages of processing whole blood into the studied products. Each of the three plasma products will have the same tests performed at T0 (within one week of collection) and T1 (after one year of routine storage).

**Laboratory Testing**

Albumin$^h$ and fibrinogen$^i$ levels and measurement of COP$^j$ were performed at The Ohio State University Veterinary Medical Center Clinical Pathology Laboratory that participates in the
annual Veterinary Laboratory Association Quality Assurance Program (Figures 2.1 and 2.2). The albumin was measured using a colorimetric assay in which cationic albumin binds with anionic dye bromocresol green to form a blue-green complex. The color intensity produced is directly proportional to the concentration of albumin and is measured photometrically by the analyzer. The fibrinogen assay is based on the Clauss methodology, in which plasma is allowed to clot in the presence of high concentrations of thrombin. The analyzer recognizes that the fibrin clot has formed when the optical density of the sample mixture has exceeded a specific threshold. The COP assay is based on water and diffusible solute particle movement through a synthetic semi-permeable membrane from a reference chamber to the sample chamber. When equilibrium is reached between the two chambers the pressure is measured by an electrical pressure transducer. Quality control is performed each time a sample is requested for COP and daily for albumin and fibrinogen assays.

Additional aliquots were shipped overnight on dry ice to the Comparative Coagulation Laboratory at Cornell University according to the laboratory recommendations for measurement of coagulation factors II, V, VII, VIII, IX, and X as previously reported (Figures 2.1 and 2.2). An ELISA with monoclonal anti-canine vWF antibodies was used to measure the concentration of von Willebrand factor. The laboratory is accredited by the American Association of Veterinary Laboratory Diagnosticians and regularly undergoes proficiency testing.

Statistical Analysis

All analyses were performed by a statistical software package. Data were assessed for normality via the D’Agostino-Pearson test. Descriptive statistics are presented as mean (± standard
deviation) or median (95% confidence interval). The outliers were identified as defined by Tukey’s method (a value lower than the lowest interquartile minus three times the interquartile or greater than the upper interquartile plus three times interquartile) and removed. Analysis of variance for repeated measures with Bonferroni correction was then performed within products between the two time points (i.e. CRYO T0 and CRYO T1) and between products at each time point (i.e. CRYO T0 and FFP T0; CPP T1 and FFP T1), with a statistical significance set at p = 0.05.

2.4 Results

Animals

All dogs used for this study were non-Greyhounds and deemed healthy on routine pre-donation screening. The dogs included 5 (50%) mixed breed dogs, 2 (20%) German shepherd dog, 1 (10%) Belgian malinois, 1 (10%) American bulldog and 1 (10%) Pit bull. There were 5 (50%) female spayed dogs and 5 (50%) male castrated dogs. The mean age of the dogs was 3.4 years (± 1.7).

Albumin and Colloid Osmotic Pressure – T0

The mean albumin levels were 28.9 g/L (± 0.5) in FFP, 31.7 g/L (± 0.7) in CPP, and 23.1 g/L (± 1.3) in CRYO (Table 2.1, Figure 2.4). The albumin levels were higher in CPP compared to FFP (p = 0.0001), higher in CPP compared to CRYO (p = 0.002), and higher in FFP compared to CRYO (p = 0.006). The mean COP in FFP was 12.73 mmHg (± 0.31), in CPP 14.5 mmHg
and in CRYO 9.8 mmHg (± 0.74) (Table 2.1). The COP was higher in CPP than in FFP (p = 0.0326), higher in CPP than in CRYO (p = 0.0068), and higher in FFP than in CRYO (p = 0.0098).

<table>
<thead>
<tr>
<th></th>
<th>FFP</th>
<th>CPP</th>
<th>CRYO</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fibrinogen (g/L)</strong></td>
<td>1.19 (0.98-1.40)(^a,b)</td>
<td>0.53 (0.05)(^c)</td>
<td>3.46 (2.65-4.27)</td>
<td>1.00-3.84</td>
</tr>
<tr>
<td><strong>Factor II (%)</strong></td>
<td>120.3 (109.1-131.6)</td>
<td>123.22 (8.29)</td>
<td>110.56 (8.38)</td>
<td>50-150%</td>
</tr>
<tr>
<td><strong>Factor V (%)</strong></td>
<td>113.8 (3.83)</td>
<td>118 (5.08)</td>
<td>88.3 (4.57)(^b,c)</td>
<td>50-150%</td>
</tr>
<tr>
<td><strong>Factor VII (%)</strong></td>
<td>118.8 (6.51)</td>
<td>107.5 (11.57)</td>
<td>116.2 (8.29)</td>
<td>50-150%</td>
</tr>
<tr>
<td><strong>Factor VIII (%)</strong></td>
<td>86 (7.98)(^a,b)</td>
<td>22.2 (2.37)(^c)</td>
<td>427 (95.4)</td>
<td>50-200%</td>
</tr>
<tr>
<td><strong>Factor IX (%)</strong></td>
<td>84.7 (5.92)</td>
<td>67.9 (2.96)(^a,c)</td>
<td>131.9 (21.87)</td>
<td>50-150%</td>
</tr>
<tr>
<td><strong>Factor X (%)</strong></td>
<td>145 (13.4)</td>
<td>121.8 (10.9)</td>
<td>125 (11.5)</td>
<td>80-175%</td>
</tr>
<tr>
<td><strong>vWF (%)</strong></td>
<td>130.7 (6.12)(^a,b)</td>
<td>22.5 (1.88)(^c)</td>
<td>504.7 (41.39)</td>
<td>70-180%</td>
</tr>
</tbody>
</table>

Table 2.1. Mean levels and standard error or median and 95% confidence interval for each plasma product at T0. FFP = fresh frozen plasma; CPP = cryopoor plasma; CRYO = cryoprecipitate, vWF = von Willebrand factor; COP = colloid osmotic pressure.

\(^a\)FFP and CPP are significantly different (p < 0.05)

\(^b\)FFP and CRYO are significantly different (p < 0.05)

\(^c\)CPP and CRYO are significantly different (p < 0.05)

**Coagulation Factors – T0**

After exclusion of 3 outliers, the median (95% confidence interval) value for fibrinogen level in FFP was 1.19 g/L (0.98-1.40) and in CRYO was 3.46 g/L (2.65-4.27) (Table 2.1, Figure 2.4). The mean fibrinogen level for CPP was 0.53 g/L (± 0.05) (Table 2.1, Figure 2.4). Fibrinogen levels were higher in CRYO than in FFP (p = 0.0004), higher in CRYO than in CPP (p = 0.0002), and higher in FFP than in CPP (p = 0.0002).
After removal of 1 outlier, the median (95% confidence interval) value for the level of factor II in FFP was 120.33% (109.07-131.60), with a mean level for CPP of 123.22% (± 8.29) and CRYO 110.56% (± 8.38) (Table 2.1). There was no significant difference in factor II levels between any of the products.

The mean value for the level of factor V in FFP was 113% (± 3.83), in CPP 118% (± 5.08), and in CRYO 88.3% (± 4.56) (Table 2.1). The factor V levels were lower in CRYO than in FFP (p = 0.018), lower in CRYO than in CPP (p = 0.004), and not significantly different between FFP and CPP (p = 1.0).

The mean values for factor VII levels were 118.8% (± 6.51) in FFP, 107.5% (± 11.57) in CPP, and 116.2% (± 8.29) in CRYO (Table 2.1). There was no significant difference in factor VII levels between any of the products.

The mean value for factor VIII level in FFP was 86.0% (± 7.98), in CPP 22.2% (± 2.37) and in CRYO 427.0% (± 95.4) (Table 2.1, Figure 2.4). Factor VIII levels were higher in CRYO compared to FFP (p = 0.012), higher in CRYO compared to CPP (p = 0.006), and higher in FFP compared to CPP (p = < 0.0001).

The mean value for factor IX level in FFP was 84.7% (± 5.92), in CPP 67.9% (± 2.95), and in CRYO 131.9% (± 21.87) (Table 2.1). The factor IX levels were lower in CPP than in FFP (p = 0.036), lower in CPP than in CRYO (p = 0.037), and not significantly different in FFP than in CRYO (p = 0.071).
The mean value for factor X level in FFP was 145.0% (±13.4), in CPP 121.8% (±10.9), and in CRYO 125.0% (±11.5). There was no significant difference in factor X levels between any of the products.

The mean value for vWf level in FFP was 130.7% (6.12), in CPP 22.5% (1.88) and in CRYO 504.7% (41.39) (Figure 2.4). The vWf levels were significantly different between all 3 products (p < 0.001 for all 3).

Figure 2.4. Levels measured for each unit of fresh frozen plasma (FFP), cryoprecipitate (CRYO), and cryopoor plasma (CPP). A. Albumin levels. Y-axis represented g/L. B. von Willebrand factor levels. Y-axis represents %. C. Factor VIII levels. Y-axis represents %. D. Fibrinogen levels. Y-axis represents g/L. Mean levels and standard deviation are reported for A, B, and C. Mean and 95% confidence interval are reported for D.
After removal of 1 outlier, the mean albumin level for CRYO was 24.33 g/L (±1.28). The mean albumin levels were 30.33 g/L (±0.50) in FFP and 32.00 g/L (±0.62) in CPP (Table 2.2). The albumin levels were higher in CPP compared to FFP (p = 0.0106), higher in CPP compared to CRYO (p = 0.004), and higher in FFP compared to CRYO (p = 0.0055) (Table 2.2). These differences were statistically significant, as they were at T0.

<table>
<thead>
<tr>
<th>Plasma Product</th>
<th>Mean (±SEM)</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen (g/L)</td>
<td>1.24 (0.98-1.48)</td>
<td>1.00-3.84</td>
</tr>
<tr>
<td>Factor II (%)</td>
<td>95.00 (±3.33)</td>
<td>50-150%</td>
</tr>
<tr>
<td>Factor V (%)</td>
<td>88.00 (±4.07)</td>
<td>50-150%</td>
</tr>
<tr>
<td>Factor VII (%)</td>
<td>78.56 (±1.47)</td>
<td>50-150%</td>
</tr>
<tr>
<td>Factor VIII (%)</td>
<td>75.86 (±4.83)</td>
<td>50-200%</td>
</tr>
<tr>
<td>Factor IX (%)</td>
<td>83.00 (±7.73)</td>
<td>50-150%</td>
</tr>
<tr>
<td>Factor X (%)</td>
<td>80.75 (±5.21)</td>
<td>80-175%</td>
</tr>
<tr>
<td>vWF (%)</td>
<td>138.50 (±8.36)</td>
<td>70-180%</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>30.33 (±0.50)</td>
<td>20-25</td>
</tr>
</tbody>
</table>

Table 2.2. Mean levels and standard error or median and 95% confidence interval for each plasma product at T1. FFP = fresh frozen plasma; CPP = cryopoor plasma; CRYO = cryoprecipitate, vWF = von Willebrand factor; COP = colloid osmotic pressure. 

<table>
<thead>
<tr>
<th>Plasma Product</th>
<th>Mean (±SEM)</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>COP (mmHg)</td>
<td>15.34 (±0.29)</td>
<td>20-25</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>30.33 (±0.50)</td>
<td>31-43</td>
</tr>
</tbody>
</table>

After removal of 2 outliers, the mean COP in CRYO was 11.17 mmHg (±0.34) (Table 2.2). After removal of 1 outlier, the mean COP in FFP was 15.34 mmHg (±0.29) (Table 2.2). The mean COP in CPP was 17.51 mmHg (±0.52) (Table 2.2). The COP was higher in CPP than in FFP (p =
0.0283), higher in CPP than in CRYO (p = 0.0005), and higher in FFP than in CRYO (p = 0.0007). These differences were statistically significant, as they were at T0.

Coagulation Factors – T1

The median (95% confidence interval) value for fibrinogen level in FFP was 1.24 g/L (0.98-1.48) and in CRYO was 3.32 g/L (2.4-4.22) (Table 2.2). The mean fibrinogen level for CPP was 54.29 g/L (±5.46) (Table 2.2). Fibrinogen levels were higher in CRYO than in FFP (p = 0.006), higher in CRYO than in CPP (p = 0.0044), and higher in FFP than in CPP (p = 0.0015). These differences were statistically significant, as they were at T0.

After removal of 1 outlier, the mean value for the level of factor II in CPP was 95.11% (±5.18) (Table 2.2). The mean factor II values were 95.00% (±3.33) in FFP and 72.33% (±4.88) in CRYO (Table 2.2). The factor II levels were lower in CRYO than in FFP (p = 0.0247), lower in CRYO than CPP (p = 0.0678), and higher in CPP than FFP (p = 1.0). There was no significant difference in factor II levels between any of the products at T0.

The mean value for the level of factor V in FFP was 88.00% (±4.07), in CPP 93.50% (±2.69), and in CRYO 64.25% (±3.13) (Table 2.2). The factor V levels were lower in CRYO than in FFP (p = 0.0005), lower in CRYO than in CPP (p = 0.0006), and not significantly different between FFP and CPP (p = 0.2876). The statistical significance, or lack thereof, was the same as at T0.
The mean values for factor VII levels were 69.13% (±5.03) in CRYO and 79.19% (±6.79) in CPP (Table 2.2). After removal of 2 outliers, the mean level of factor VII in FFP was 78.56% (±1.47) (Table 2.2). There was no significant difference in factor VII levels between any of the products, as was found at T0.

The mean value for factor VIII level in FFP was 75.86% (±4.83) (Table 2.2). After removal of 1 outlier, the mean value for factor VIII level in CPP was 27.00% (±0.87) (Table 2.2). After removal of 2 outliers, the mean value for factor VIII level in CRYO was 123.71% (±8.44). Factor VIII levels were higher in CRYO compared to FFP (p = 0.0059), higher in CRYO compared to CPP (p < 0.0001), and higher in FFP compared to CPP (p = 0.001). These differences were statistically significant, as they were at T0.

The mean value for factor IX level in FFP was 83.00% (±7.73), in CPP 47.00% (±4.70), and in CRYO 119.8% (±24.21) (Table 2.2). The factor IX levels were lower in CPP than in FFP (p = 0.001), lower in CPP than in CRYO (p = 0.0418), and not significantly different in FFP than in CRYO (p = 0.2683). The statistical significance, or lack thereof, was the same as at T0.

The mean value for factor X level in FFP was 80.75% (±5.21), in CPP 84.50% (±5.71), and in CRYO 70.75% (±4.04) (Table 2.2). There were no significant differences in factor X levels between any of the products, as was found at T0.

The mean value for vWF level in FFP was 138.50% (±8.36) and in CRYO was 521.25% (±42.23) (Table 2.2). After removal of 2 outliers, the mean value for vWF level in CPP was 22.75% (±0.67)
The vWF levels were significantly different between all 3 products (p < 0.001 for all 3), as seen at T0.

<table>
<thead>
<tr>
<th>Product</th>
<th>Variable</th>
<th>T0</th>
<th>T1</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFP</td>
<td>Fibrinogen (g/L)</td>
<td>1.19 (±0.09)</td>
<td>1.24 (0.98-1.48)</td>
<td>0.077</td>
</tr>
<tr>
<td></td>
<td>Factor II (%)</td>
<td>120.33 (±4.88)</td>
<td>95.00 (±3.33)</td>
<td>0.0016</td>
</tr>
<tr>
<td></td>
<td>Factor V (%)</td>
<td>113.63 (±4.81)</td>
<td>88.00 (±4.07)</td>
<td>0.0003</td>
</tr>
<tr>
<td></td>
<td>Factor VII (%)</td>
<td>112.88 (±6.19)</td>
<td>78.56 (±4.17)</td>
<td>0.0023</td>
</tr>
<tr>
<td></td>
<td>Factor VIII (%)</td>
<td>87.29 (±9.32)</td>
<td>75.86 (±4.83)</td>
<td>0.3463</td>
</tr>
<tr>
<td></td>
<td>Factor IX (%)</td>
<td>84.7 (±5.92)</td>
<td>83.00 (±7.73)</td>
<td>0.7516</td>
</tr>
<tr>
<td></td>
<td>Factor X (%)</td>
<td>145 (±13.4)</td>
<td>80.75 (±5.21)</td>
<td>0.0032</td>
</tr>
<tr>
<td></td>
<td>vWF (%)</td>
<td>126.63 (±7.81)</td>
<td>138.50 (±8.36)</td>
<td>0.020</td>
</tr>
<tr>
<td></td>
<td>COP (mmHg)</td>
<td>12.40 (±0.35)</td>
<td>15.34 (±0.29)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td></td>
<td>Albumin (g/L)</td>
<td>28.78 (±0.55)</td>
<td>30.33 (±0.50)</td>
<td>0.0002</td>
</tr>
<tr>
<td>CRYO</td>
<td>Fibrinogen (g/L)</td>
<td>345.85 (±33.21)</td>
<td>3.32 (2.4-4.22)</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Factor II (%)</td>
<td>110.556 (±8.38)</td>
<td>72.33 (±4.88)</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Factor V (%)</td>
<td>86.75 (±5.60)</td>
<td>64.25 (±3.13)</td>
<td>0.0066</td>
</tr>
<tr>
<td></td>
<td>Factor VII (%)</td>
<td>116.38 (±10.33)</td>
<td>69.13 (±5.03)</td>
<td>0.0076</td>
</tr>
<tr>
<td></td>
<td>Factor VIII (%)</td>
<td>408.0 (±120.18)</td>
<td>123.71 (±8.44)</td>
<td>0.0477</td>
</tr>
<tr>
<td></td>
<td>Factor IX (%)</td>
<td>131.9 (±21.87)</td>
<td>119.8 (±24.21)</td>
<td>0.3167</td>
</tr>
<tr>
<td></td>
<td>Factor X (%)</td>
<td>125 (±11.5)</td>
<td>70.75 (±4.04)</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td>vWF (%)</td>
<td>489.00 (±54.83)</td>
<td>521.25 (±42.23)</td>
<td>0.1153</td>
</tr>
<tr>
<td></td>
<td>COP (mmHg)</td>
<td>8.84 (±0.48)</td>
<td>11.17 (±0.34)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>Albumin (g/L)</td>
<td>23.33 (±1.14)</td>
<td>24.33 (±1.28)</td>
<td>0.125</td>
</tr>
<tr>
<td>CPP</td>
<td>Fibrinogen (g/L)</td>
<td>52.85 (±5.31)</td>
<td>54.29 (±5.46)</td>
<td>0.283</td>
</tr>
<tr>
<td></td>
<td>Factor II (%)</td>
<td>123.22 (±8.29)</td>
<td>95.11 (±5.18)</td>
<td>0.0014</td>
</tr>
<tr>
<td></td>
<td>Factor V (%)</td>
<td>115.00 (±5.47)</td>
<td>93.50 (±2.69)</td>
<td>0.0006</td>
</tr>
<tr>
<td></td>
<td>Factor VII (%)</td>
<td>101.75 (±13.04)</td>
<td>79.19 (±6.79)</td>
<td>0.1846</td>
</tr>
<tr>
<td></td>
<td>Factor VIII (%)</td>
<td>25.29 (±2.17)</td>
<td>27.00 (±0.87)</td>
<td>0.4337</td>
</tr>
<tr>
<td></td>
<td>Factor IX (%)</td>
<td>67.9 (±2.96)</td>
<td>47.00 (±4.70)</td>
<td>0.0015</td>
</tr>
<tr>
<td></td>
<td>Factor X (%)</td>
<td>121.8 (±10.9)</td>
<td>84.50 (±5.71)</td>
<td>0.0080</td>
</tr>
<tr>
<td></td>
<td>vWF (%)</td>
<td>23.00 (±2.46)</td>
<td>22.75 (±0.67)</td>
<td>0.9240</td>
</tr>
<tr>
<td></td>
<td>COP (mmHg)</td>
<td>14.3 (±0.79)</td>
<td>17.51 (±0.52)</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>Albumin (g/L)</td>
<td>31.44 (±0.68)</td>
<td>32.00 (±0.62)</td>
<td>0.051</td>
</tr>
</tbody>
</table>

Table 2.3. Mean and standard error or median and 95% confidence interval for T0 and T1, with p values. FFP = fresh frozen plasma; CRYO = cryoprecipitate; CPP = cryopoor plasma; COP = colloid osmotic pressure; vWF = von Willebrand factor.
Comparison of T0 to T1

The pairwise comparison of the measured variables at T0 and T1 are shown in Table 2.3. There was a significant decrease in the following: factor II, V and X in all 3 products, factor VII in FFP and CRYO, factor VIII in CRYO, and factor IX in CPP (Table 2.3). There was a significant increase in COP in all 3 products and vWf and albumin in FFP (Table 2.3). No other significant changes in the measured variables were seen (Table 2.3).

2.5 Discussion

To the author’s knowledge, this study is the first to report the albumin, COP, and coagulation factor levels in the canine plasma fractionation byproducts CPP and CRYO, compared to their source FFP. Fresh frozen plasma is partially thawed in order to separate out factors based on their different freezing points, which should in theory separate fibrinogen, factor VIII and vWf in CRYO, leaving the remaining coagulation factors in higher concentrations in CPP.32 Our findings confirmed that our method used to process FFP into CRYO does successfully produce the expected product.

Deficits of one or multiple coagulation factors are common in dogs, with diseases such as disseminated intravascular coagulopathy, hemophilia A (deficiency of factor VIII), hemophilia B (deficiency of factor IX), von Willebrand’s disease, hepatic disease or anticoagulant rodenticide toxicity.30,48,49 A goal of this study was to determine the vWf and coagulation factor content of various plasma products that may be used to treat some of these conditions.
In our study, CRYO contains higher levels of factor VIII, vWF, and fibrinogen as compared to FFP and CPP. These factor levels were also higher in FFP as compared to CPP. There is limited data on the use of CRYO in veterinary medicine. One study found that greater increases in vWF levels were achieved with cryoprecipitate than FFP in dogs with von Willebrand’s disease.\textsuperscript{30} That study also documented similar increases in factor VIII with infusions of FFP and CRYO in dogs with hemophilia A. Another study documented shortened mean buccal mucosal bleeding time and increases in vWF levels in Doberman Pinschers with von Willebrand’s disease that were treated with CRYO.\textsuperscript{33} In that study, administration of FFP increased the plasma vWF but did not shorten the buccal mucosal bleeding time. Neither of these studies, however, investigated levels of vWF and factor VIII in the blood products themselves.

Our study also found a higher fibrinogen level in CRYO than in FFP. In humans, CRYO is recommended for fibrinogen replacement.\textsuperscript{34} A recent human clinical trial evaluated the feasibility of treating patients with significant hemorrhage secondary to trauma with CRYO shortly after presentation to provide early fibrinogen support.\textsuperscript{35} This study found that the use of fibrinogen supplementation with CRYO is feasible in trauma patients, however no 28-day mortality difference was observed and further investigation on outcome is warranted. Another study retrospectively investigated the administration of CRYO on human trauma patients and found that it was uncommonly administered (3.6% of patients) and no mortality difference was observed in patients with hypofibrinogenemia who were treated with CRYO versus those who did not receive CRYO.\textsuperscript{36} In contrast, the MATTERs II Study found that concurrent use of CRYO may independently improve the survival benefit of tranexamic acid in trauma patients requiring red blood cell transfusions.\textsuperscript{37} No clinical study has investigated the impact of CRYO on fibrinogen
levels on canine patients. However, our study suggests that CRYO can be used if blood products containing high levels of fibrinogen are needed in canine patients.

There is no previously published data on CPP in veterinary medicine. In humans, CPP is reported to have reduced levels of vWF, fibrinogen, and factor VIII. Due to high levels of a disintegrin and metalloproteinase thrombospondin type 1 motif, member 13 (ADAMTS13) in CPP, its use in human medicine is primarily for treatment of thrombotic thrombocytopenia purpura, a condition not reported in veterinary medicine, in lieu of FFP for plasma exchange. Cryopoor plasma has been also reported for use in lieu of FFP for the treatment of disseminated intravascular coagulation associated with pancreonecrosis and generalized peritonitis in humans. In veterinary medicine, CPP is listed for use in treatment of vitamin K antagonist rodenticide toxicity, although there is no published data to support its use for this condition. Our study found that CPP contains statistically similar levels of vitamin K dependent factors II, VII, and X compared to FFP and CRYO. This data also supports the use of CPP as a treatment for anticoagulant rodenticides, although in-vivo studies comparing the efficacy of CPP to FFP to treat anticoagulant rodenticide toxicity are warranted. However, it contains significantly less factor IX than FFP and CRYO, suggesting that CRYO or FFP should be preferred over CPP for treatment of hemophilia B. Reports of factor IX levels in various plasma products are scarce in human medicine and not available in veterinary medicine. One study reported significantly higher levels of factor IX in FFP than CPP, however factor IX levels were not measured in CRYO. Another study also found lower factor IX levels in CPP than FFP and higher levels of factor IX in CRYO than FFP, consistent with our findings. The division of factors in plasma fractionation products is due to the difference in freezing points of the various factors. To the authors’ knowledge, the freezing points of canine coagulation factors have not been published.
Decreased albumin level and COP are common findings in critically ill dogs with conditions such as septic peritonitis, hemorrhage, and renal or intestinal losses.\textsuperscript{5–9} Hypoalbuminemia is associated with a variety of negative effects, including decreased survival, poor healing and changes to drug binding capacity in both human and veterinary studies.\textsuperscript{5,6,8,22} Additionally, albumin is responsible for 70-80\% of COP, thus hypoalbuminemia is often associated with a decreased COP and increased risk of edema.\textsuperscript{1} In humans, albumin replacement has been shown to be beneficial in septic patients.\textsuperscript{13,14} The human SAFE study compared the use of a 4\% albumin solution with normal saline for fluid resuscitation in patients in the intensive care unit.\textsuperscript{14} Analysis of the septic subgroup of the SAFE study showed improvement of survival in the group treated with 4\% albumin. It is of note that the percentage of albumin found in the CPP in our study was similar to the 4\% used in the SAFE study. The ALBIOS study found improved survival in septic shock patients treated with 20\% albumin and crystalloids as compared to patients treated with crystalloids alone.\textsuperscript{13} Species-specific albumin replacement is the most logical replacement product for hypoalbuminemia, but accomplishing this goal in veterinary medicine is difficult due to the lack of readily available, canine-specific albumin concentrate.\textsuperscript{15} As such, veterinary clinicians may have to rely on the administration of FFP, 25\% human serum albumin or synthetic colloids such as hydroxyethylstarches (HES). All three may be used to increase COP, however synthetic colloids do not contain albumin. However, use of 25\% human serum albumin and HES is associated with various risks, many of which have only recently been recognized and investigated in veterinary medicine.\textsuperscript{15,52} Because of the large volume of FFP needed to treat hypoalbuminemia (up to 45 mL/kg to raise the albumin by 10 g/L depending the formula used), there is increased concern for volume overload.\textsuperscript{23,53} Additionally, the cost of FFP transfusions for oncotic or albumin support is often too high to be a feasible treatment option for this condition for most pet owners. Some veterinary references suggest the use of CPP for albumin replacement,
although there is no published supporting data for this claim. Our study found that albumin levels and COP measurement were significantly higher in CPP compared to FFP and CRYO. It appears that the process of separating CRYO and CPP from FFP also separates albumin, probably due to its freezing point temperature, that allows its concentration with CPP. Additionally, the removal of the volume of albumin-deficient CRYO from FFP to make CPP results in a more concentrated albumin product. This suggests that CPP can be a less expensive and more effective canine-specific albumin replacement than FFP. The use of CPP for albumin replacement warrants further investigation.

When comparing T0 to T1, COP was significantly increased in all 3 products, however the albumin was only increased in 1 product at T1, which suggests that other contributors such as globulins may have changed with storage and impacted COP. Globulin was not measured in our study. There was a significant decrease in labile factor V in all 3 products, which is consistent with what is previously published in human plasma products, however a decrease in factor V with storage has not previously been reported in veterinary medicine. Routine storage has also been previously documented in veterinary medicine to cause a decrease in fibrinogen, vWF, and factors IX and X, which was seen in many of the products in our study. Although a statistically significant decrease in multiple coagulation factors was seen, the cause and the clinical significance of this decrease are unclear. Based on previously published doses of FFP for treatment of vWF disease and hemophilias A and B, as well as a 2001 study evaluating the effect of storage on multiple coagulation factors in FFP, the post-storage decrease in the levels of many of the coagulation factors in our study is likely not clinically significant. Variation in laboratory techniques cannot be ruled out as a possible contributor to the T0 and T1 differences, although both laboratories used practice routine quality control and frequent calibration of
equipment. Inadequate mixing of samples prior to testing at either time point could have resulted in inconsistent results, although efforts were made to thoroughly agitate the samples prior to testing.

There were some limitations in this study. Although Greyhounds are frequently used for blood donation, we elected to use non-Greyhounds to evaluate a more diverse breed population that may be more representative of a community-based blood bank. Use of Greyhound donors may have revealed different results. It has been documented that albumin levels in Greyhound are similar to non-Greyhound dogs. It has also been documented that Greyhounds have lower globulin levels, a lower total protein and lower fibrinogen levels than non-Greyhound dogs.

Although some standard protocols for processing of veterinary plasma products exist, it is unclear if those results can be extrapolated to a different blood bank that may use a different protocol, and therefore only represent the standards in our blood bank. Individual variation in levels of measured values in each product were seen, although these differences were not statistically significant. Lastly, our study does not provide any information about the long-term storage of CPP, although our blood bank recommends a shelf life for up to 6 years at standard storage conditions.

The data in this study supports the use of CRYO for treatment of deficiencies in von Willebrand factor, fibrinogen, factor VIII, and factor IX, as well as the use of CPP for treatment of hypoalbuminemia and low COP and vitamin-K dependent factor deficiency. Further investigation of the clinical use of CPP in these situations is warranted. This study also found that the majority of measured variables maintained likely clinically significant levels after 1 year of routine storage.
Chapter 3: Clinical Use of Cryopoor Plasma Continuous Rate Infusion in Critically Ill, Hypoalbuminemic Canine Patients

3.1 Abstract

Objective—To evaluate the potential impact of CPP continuous rate infusion (CRI) on albumin and COP in critically ill canine patients with documented hypoalbuminemia and low COP.

Design—Retrospective clinical study.

Setting—University teaching hospital.

Animals—Ten hypoalbuminemic, critically ill canine patients in the Intensive Care Unit

Interventions—Retrospective evaluation of 10 hypoalbuminemic dogs receiving a CPP infusion for albumin replacement. Factors including signalment, survival predictor index (SPI2) score, disease process, duration of infusion, total volume, dose, and rate of CPP, crystalloids, and hydroxyethylstarch (HES) administered during the CPP infusion, pre- and post-infusion albumin and colloid osmotic pressure (COP), duration of hospitalization, and outcome were evaluated.

Measurements and Main Results—Seven of the dogs were septic, with 29% of those being in septic shock and 71% having septic peritonitis. The mean pre- and post-infusion albumin were 15.4 g/L (±3.9) and 21 g/L (±2.5), respectively, and were significantly different (p = 0.011). The median pre- and mean post-infusion COP were 8.6 mmHg (4.9-9.7) and 10.5 mmHg (±1.5) respectively, and were significantly different (p = 0.0039). The median duration of infusion was 16 hours (11-121). The mean rate of CPP administered was 1.8 mL/kg/hr (±0.6), the mean rate of
crystalloid administered was 0.8 mL/kg/hr (±0.9), and the mean rate of HES administered was 1.2 mL/kg/hr (±0.9). Delta albumin was positively correlated with CPP mL/kg/hr (p = 0.0004), while delta COP was positively correlated with HES mL/kg/hr (p = 0.0128). Mean duration of hospitalization was 8.6 days (±3.9). No side effects of CPP infusion were reported. Age was the only factor associated with survival (p = 0.033) with non-survivors more likely to be older. 30% of patients died, 30% of patients were euthanized, and 40% of patients survived to discharge. All discharged patients survived to 90 days post-discharge.

**Conclusions**— The dose of CPP administered was correlated with an increase in albumin, but not with an increase in COP. Given the concurrent administration of HES, which has a higher oncotic pressure than CPP, it is possible that the impact of CPP on COP was overshadowed by the influence of HES. CPP may be a valid option for treatment of hypoalbuminemia and low COP, however further clinical studies are warranted regarding dose, safety and impact on outcome.

### 3.2 Introduction

Hypoalbuminemia and low colloid osmotic pressure (COP) are common findings in critically ill dogs. Hypoalbuminemia is associated with various negative effects, including increased mortality, decreased tissue healing, and impacts on the binding capacity of drugs. Given the significant contribution of albumin to COP, hypoalbuminemia is often associated with decreased COP and increased risk of edema. Synthetic colloids such as hydroxyethylstarches (HES) may be given to help raise COP, however these have no positive impact on albumin levels and can be associated with risks such as increased potential for AKI in septic patients. Natural colloids are potentially beneficial because they contain albumin, although options are limited in veterinary medicine. An albumin concentrate, 25% human serum albumin (HSA) can be administered to
improve albumin levels, however HSA may be associated with potentially life-threatening hypersensitivity reactions and multiple transfusions cannot be administered. Species-specific albumin is an ideal option, as it greatly reduces the risk of transfusion reactions. Fresh frozen plasma (FFP) may be given to provide albumin and oncotic support and given the large volume is needed to treat hypoalbuminemia, it may be too expensive to be a feasible option for many dog owners. Our study, reported in Chapter 2, found that cryopoor plasma (CPP) contains significantly more albumin and has a higher COP than FFP, suggesting that it may be a potential treatment option for hypoalbuminemia. A benefit of CPP is that it is approximately half the cost of FFP by volume and thus may offer a more cost effective treatment option for hypoalbuminemia. Additionally, targeted transfusion would ensure better blood product stewardship and would help maximize the impact of each blood donation. To the authors’ knowledge, the clinical use of CPP in hypoalbuminemic patients has not been evaluated.

The objective of this study was to compare pre and post CPP transfusion albumin and COP in critically ill canine patients with hypoalbuminemia and low COP. We hypothesized that the albumin and COP would increase with the administration of CPP.

3.3 Materials and Methods

Study Population and Clinical Evaluation

The records of The Ohio State University College of Veterinary Medical Center Blood Bank were searched for patients that received canine CPP between July 2013 and December 2015. The corresponding medical records for these patients were then reviewed. Inclusion criteria were as follows: (1) Canine patients with documented hypoalbuminemia and/or low COP (2) Canine
patients receiving CPP. Exclusion criteria were: (1) Patients without documented hypoalbuminemia and/or low COP (2) Patients receiving CPP for reasons other than hypoalbuminemia or low COP (such as anticoagulant rodenticide toxicity).

Data retrieved from the medical records included signalment, body weight (kilograms) within 24 hours of transfusion, underlying disease process(es), albumin and COP at the start of CPP transfusion, albumin and COP at the end of CPP transfusion, total volume, total dose (mL/kg) and rate (mL/kg/hr) of CPP transfused, duration of CPP transfusion, total volume, total dose (mL/kg) and total rate (mL/kg/hr) of crystalloids and HES given concurrent to CPP transfusion, reported side effects, duration of hospitalization, and outcome of patient (death, euthanasia, discharge, and 30 day survival). The delta albumin and COP were calculated by subtracting the pre-infusion value from the post-infusion value. The survival prediction index score (SPI2) was calculated using values obtained prior to the first infusion\(^5\) (Appendix A).

Statistical Analysis
All analyses were performed by a statistical software package\(^6\). Mann-Whitney U test was used to compare survivors and non-survivors for age, body weight, SPI2 score, mean arterial blood pressure, respiratory rate, creatinine, packed cell volume, pre and post infusion albumin and COP, delta albumin and COP, total volume CPP administered, CPP dose (mL/kg), and CPP rate (mL/kg/hr), total crystalloid administered, total colloid administered, total Jackson-Pratt drain production, and duration of hospitalization. Fisher’s Exact test was used to compare survivors and non-survivors for categorical data. Pre and post COP were compared using Wilcoxon test. Delta COP and colloid rate of administration (mL/kg/hr), delta COP and CPP rate of administration
(mL/kg/hr), pre and post albumin, and delta albumin and COP administration rate (ml/kg/hr) were
normally distributed and thus were evaluated using a paired sample t-test.

3.4 Results

Patient Population
A total of 17 dogs received CPP during the study period. Seven of those dogs were excluded
because CPP was administered for anticoagulant rodenticide toxicity. Ten dogs satisfied the
inclusion criteria. These included 6 (60%) castrated males, 3 (30%) spayed females, and 1 (10%)
intact female (Table 3.1). The mean age at the time of infusion was 7.4 years (±4.5). Breeds
represented were mixed breed (n = 5), Bernese Mountain Dog (n = 1), Rat Terrier (n = 1),
Siberian Husky (n = 1), Rottweiler (n = 1), and Welsh Corgi (n = 1) (Table 3.1). The mean SPI2
score was 0.66 (±0.13).

Seven (70%) of patients were septic; of these 29% were in septic shock and 71% had septic
peritonitis. Causes of sepsis included dehiscence of an enterotomy or intestinal resection and
anastomosis (3/7), septic uroabdomen secondary to bladder rupture (1/7), septic bile peritonitis
secondary to a ruptured gall bladder mucocele (1/7), septic shock associated with parvoviral
infection (1/7), and septic polyarthritis (1/7) (Table 3.1). One of the patients with septic
peritonitis had concurrent protein losing enteropathy and the septic peritonitis was the result of
dehiscence of an intestinal biopsy site. Of the 3 non-septic patients, 1 had a large intra-abdominal
lipoma removed, 1 had a partial pancreatectomy for a sterile pancreatic abscess, and 1 had a liver
lobectomy for mass removal, cholecystectomy for gall bladder mucocele, left adrenalectomy and
left nephrectomy (Table 3.1).
Table 3.1. Signalment, underlying disease process, outcome, and days of hospitalization of the patients included in the study. MC = male castrated; FS = female spayed; F = intact female; RNA = intestinal resection and anastomosis; PLE = protein losing enteropathy.

**Response to Therapy**

The median total volume of CPP transfused was 31 mL/kg (12.7-217.8). The median duration of CPP continuous rate infusion (CRI) was 16 hours (11-121) (Table 3.2). Of the patients receiving concurrent isotonic crystalloids during the CPP CRI, the median total volume of isotonic
crystalloids administered was 9.4 mL/kg (0-106) (Table 3.2). Three patients did not receive isotonic crystalloids during the CPP infusion. The mean total volume of concurrent HES administered during the CPP CRI was 33.1 mL/kg (±33.3) (Table 3.2). One patient received no HES during the CPP infusion. Nine patients received HES 6% 130/0.4/9:1 and one patient received both HES 6% 130/0.4/9:1 and HES 6% 670/0.75/4:1. No transfusion reactions were reported.

<table>
<thead>
<tr>
<th>Case</th>
<th>Cryopoor Plasma (mL/kg)</th>
<th>Isotonic Crystalloids (mL/kg)</th>
<th>HES (mL/kg)</th>
<th>Total time of infusion (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>192.6</td>
<td>106</td>
<td>103.23</td>
<td>106</td>
</tr>
<tr>
<td>Case 2</td>
<td>23.89</td>
<td>0</td>
<td>3.59</td>
<td>13</td>
</tr>
<tr>
<td>Case 3</td>
<td>15.23</td>
<td>31.25</td>
<td>23.93</td>
<td>16</td>
</tr>
<tr>
<td>Case 4</td>
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<td>0</td>
<td>11.59</td>
<td>16</td>
</tr>
<tr>
<td>Case 5</td>
<td>217.84</td>
<td>5.51</td>
<td>81.44</td>
<td>121</td>
</tr>
<tr>
<td>Case 6</td>
<td>32.97</td>
<td>0</td>
<td>24.73</td>
<td>18</td>
</tr>
<tr>
<td>Case 7</td>
<td>37.59</td>
<td>30.17</td>
<td>21.72</td>
<td>24</td>
</tr>
<tr>
<td>Case 8</td>
<td>12.73</td>
<td>13.28</td>
<td>34.13</td>
<td>12</td>
</tr>
<tr>
<td>Case 9</td>
<td>40.15</td>
<td>31.39</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Case 10</td>
<td>26.12</td>
<td>1.21</td>
<td>26.67</td>
<td>11</td>
</tr>
</tbody>
</table>

Table 3.2. Volume and type of fluids administered concurrent to CPP infusion. *median and range; ^ mean and standard error. HES = hydroxyethylstarch.

Table 3.3 shows the mean and standard error to median and range for the variables evaluated. The mean albumin prior to CPP infusion was 15.4 g/L (±3.9). The mean albumin after CPP infusion was 21 g/L (±2.5). The median COP prior to CPP infusion was 8.6 mmHg (4.9-9.7). The mean COP after CPP infusion was 10.5 mmHg (±1.5).
The pre and post albumin (p = 0.011) and pre and post COP (p = 0.0039) were significantly different. Delta albumin and CPP rate of administration (mL/kg/hr) (p = 0.0004) and delta COP and HES rate of administration (mL/kg/hr) (p = 0.0128) were significantly associated. Delta COP and CPP rate of administration were not significantly associated (p = 0.1852).

<table>
<thead>
<tr>
<th>Factor Evaluated</th>
<th>All Patients</th>
<th>Survivor</th>
<th>Non-Survivor</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>7.4 (± 4.5)</td>
<td>3.5 (0.8-6)</td>
<td>11.5 (5-14)</td>
<td>0.033</td>
</tr>
<tr>
<td>Sex (Female / Male)</td>
<td>4 / 6</td>
<td>3/1 (75%)</td>
<td>1/5 (17%)</td>
<td>0.190</td>
</tr>
<tr>
<td>SPI2 score</td>
<td>0.66 (± 0.13)</td>
<td>0.76 (0.64-0.82)</td>
<td>0.62 (0.4-0.76)</td>
<td>0.136</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>96.6 (± 28.3)</td>
<td>81 (73-96)</td>
<td>97 (70-153)</td>
<td>0.337</td>
</tr>
<tr>
<td>Respiratory rate (bpm)</td>
<td>31 (24-100)</td>
<td>42 (24-100)</td>
<td>31 (24-40)</td>
<td>0.831</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.94 (±0.41)</td>
<td>0.75 (0.6-1.6)</td>
<td>0.9 (0.5-1.7)</td>
<td>0.749</td>
</tr>
<tr>
<td>Packed cell volume (%)</td>
<td>23.3 (± 3.0)</td>
<td>24.5 (21-28)</td>
<td>23 (18-25)</td>
<td>0.284</td>
</tr>
<tr>
<td>Pre-albumin (g/L)</td>
<td>15.4 (± 3.9)</td>
<td>19.5 (11-21)</td>
<td>13 (11-17)</td>
<td>0.166</td>
</tr>
<tr>
<td>Pre-COP (mmHg)</td>
<td>8.6 (4.9-9.7)</td>
<td>8.85 (8.00-9.70)</td>
<td>7.3 (4.9-9.0)</td>
<td>0.142</td>
</tr>
<tr>
<td>Post-Albumin (g/L)</td>
<td>21 (± 2.5)</td>
<td>21 (20-26)</td>
<td>20 (17-23)</td>
<td>0.187</td>
</tr>
<tr>
<td>Post-COP (mmHg)</td>
<td>10.5 (± 1.5)</td>
<td>10.3 (9.5-13.3)</td>
<td>10.2 (8.1-12.1)</td>
<td>0.807</td>
</tr>
<tr>
<td>Delta Albumin (g/L)</td>
<td>5.2 (±3.49)</td>
<td>4.0 (0.0-9.0)</td>
<td>4.5 (3.0-11)</td>
<td>0.517</td>
</tr>
<tr>
<td>Delta COP (mmHg)</td>
<td>2.4 (1±57)</td>
<td>1.85 (0.1-4.2)</td>
<td>3.2 (1.1-4.8)</td>
<td>0.624</td>
</tr>
<tr>
<td>CPP (mL/kg)</td>
<td>31 (12.7-217.8)</td>
<td>31.8 (23.9-192.6)</td>
<td>31.0 (12.7-217.8)</td>
<td>0.831</td>
</tr>
<tr>
<td>CPP (mL/kg/hr)</td>
<td>1.8 (± 0.6)</td>
<td>1.8 (1.6-2.4)</td>
<td>1.8 (0.9-3.1)</td>
<td>0.394</td>
</tr>
<tr>
<td>Crystalloid (mL/kg)</td>
<td>9.4 (0-106)</td>
<td>20.7 (0.0-41.2)</td>
<td>9.4 (0.0-31.4)</td>
<td>0.521</td>
</tr>
<tr>
<td>Crystalloid (mL/kg/hr)</td>
<td>0.8 (± 0.9)</td>
<td>0.55 (0.00-1.26)</td>
<td>0.58 (0.00-2.41)</td>
<td>0.831</td>
</tr>
<tr>
<td>Hydroxyethylstarch (mL/kg)</td>
<td>33.1 (± 33.3)</td>
<td>24.2 (3.6-103.2)</td>
<td>24.3 (0.0-81.4)</td>
<td>0.831</td>
</tr>
<tr>
<td>Hydroxyethylstarch (mL/kg/hr)</td>
<td>1.2 (± 0.9)</td>
<td>0.9 (0.3-2.4)</td>
<td>1.1 (0.0-2.8)</td>
<td>1.00</td>
</tr>
<tr>
<td>JP drain production (mL/kg/hr)</td>
<td>0.8 (± 0.9)</td>
<td>0.43 (0.00-2.56)</td>
<td>0.49 (0.00-2.08)</td>
<td>0.831</td>
</tr>
<tr>
<td>Duration of hospitalization (days)</td>
<td>8.6 (± 3.9)</td>
<td>12.5 (6-14)</td>
<td>6.0 (2-12)</td>
<td>0.070</td>
</tr>
</tbody>
</table>

**Table 3.3.** Mean and standard error or median and range for evaluated variables in all patients. Median and range of various factors of survivors vs. non-survivors with associated p values. SPI2 = survival prediction index; COP = colloid osmotic pressure; CPP = cryopoor plasma; JP = Jackson Pratt drain.
<table>
<thead>
<tr>
<th>Case</th>
<th>Pre-CPP</th>
<th>Post-CPP</th>
<th>Pre-CPP</th>
<th>Post-CPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21</td>
<td>26</td>
<td>9.1</td>
<td>13.3</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>22</td>
<td>9.7</td>
<td>9.8</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>20</td>
<td>8.8</td>
<td>9.9</td>
</tr>
<tr>
<td>4</td>
<td>12</td>
<td>18</td>
<td>4.9</td>
<td>8.1</td>
</tr>
<tr>
<td>5</td>
<td>12</td>
<td>23</td>
<td>7.3</td>
<td>10.6</td>
</tr>
<tr>
<td>6</td>
<td>11</td>
<td>20</td>
<td>7.3</td>
<td>12.1</td>
</tr>
<tr>
<td>7</td>
<td>11</td>
<td>20</td>
<td>8.6</td>
<td>10.8</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>17</td>
<td>9</td>
<td>10.2</td>
</tr>
<tr>
<td>9</td>
<td>17</td>
<td>20</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>10</td>
<td>20</td>
<td>20</td>
<td>8</td>
<td>9.5</td>
</tr>
<tr>
<td>Mean</td>
<td>15.4 (±3.9)</td>
<td>21 (±2.5)</td>
<td>8.1 (±1.4)</td>
<td>10.5 (±1.5)</td>
</tr>
</tbody>
</table>

Table 3.4. Pre and post CPP infusion albumin and COP. Mean and standard error were reported for all data for ease. CPP = cryopoor plasma; COP = colloid osmotic pressure.

**Outcome**

Three (30%) of patients were euthanized due to concerns for quality of life, failure to respond to treatment, and financial concerns. Three patients (30%) died of cardiopulmonary arrest. One of these dogs died of a thrombotic shower to numerous organs including the kidneys and lungs, confirmed on autopsy. Autopsy was not performed on the other two dogs. Four patients (40%) were discharged from the hospital, with a mean duration of hospitalization of 8.6 days (±3.9). Of the patients that were discharged, all 4 (100%) survived to 90 days. All of the patients that survived were septic, with 57% of the septic patients surviving.

As seen in Table 3.3, survivors were found to be younger (3.5 years vs. 11.5, p = 0.033). None of the other factors that were evaluated had significant association with survival. There was a trend toward survival for female patients, although the difference was not statistically significant.
3.5 Discussion

To the authors’ knowledge, this study is the first to report the clinical use of CPP to treat hypoalbuminemia and low COP in critically ill canine patients. Our previous study reported in Chapter 2 found that CPP contains higher levels of albumin and has a higher COP than FFP, supporting its use as a natural colloid. In that study, the mean albumin and COP in CPP were significantly higher than levels in FFP, with 31.7 g/L (±0.7) in CPP compared to 28.9 g/L (±0.5) in FFP (p = < 0.001) and 14.5 mmHg (±0.65) in CPP compared 12.73 mmHg (± 0.31) in FFP (p = 0.03), respectively.

As previously discussed in Chapters 1 and 2, hypoalbuminemia and decreased COP are associated with a variety of negative effects in the critically ill patient such as increased mortality, impaired tissue healing, and decreased drug binding capacity. Colloid support may be provided in the form of synthetic or natural colloids, each of which come with unique risks and benefits. Synthetic colloids such as HES may be given to help increase COP as they are convenient, relatively inexpensive, widely available, and require no special equipment for administration, however they have no positive impact on albumin levels. In human medicine, HES are known to have significant side effects such as acute kidney injury in some patient populations, which may further compromise an already critical patient, and their use in septic patients is no longer recommended. The human Surviving Sepsis Campaign Guidelines recommend against the use of HES and suggest instead using albumin in cases of severe sepsis and septic shock. Additionally, large volumes of starches have been showed to potentially cause coagulopathies, which could also be detrimental to a patient.
An alternative to hydroxyethyl starches is natural colloids, which are beneficial because they contain albumin, however options for natural colloids are limited in veterinary medicine. 25% HSA provides a higher albumin support than plasma, depending on the dose given, however no prospective studies have yet been published on its use in critical patients and there are legitimate concerns about type I and III hypersensitivity reactions. As such, it is typically reserved for critically ill patients and repeated transfusions in a single patient are not recommended. Species-specific albumin replacement is the most logical treatment for hypoalbuminemia and is recommended for human septic patients. Clinicians desiring a canine-specific albumin product must give a canine plasma product such as FFP or CPP. Transfusion reactions from canine plasma are possible, with a low incidence, reportedly occurring in only 1-3% of patients, and reactions are often minor such as fever and pruritus. It should be noted that no transfusion reactions were reported in our study. FFP may be given to provide albumin and oncotic support, however a large volume is needed to treat hypoalbuminemia (up to 45 mL/kg to raise the albumin by 1.0 mg/dL, depending on the formula used), which may be cost prohibitive for many dog owners. Our study, reported in Chapter 2, found that CPP contains more albumin and COP than FFP, suggesting that it is a potential treatment option for hypoalbuminemia. At our blood bank, CPP is half the cost of FFP, which could make a potentially more realistic option for owners. Additionally, a more tailored use of blood products would improve blood product stewardship and could maximize the potential benefits of a single whole blood donation.

There is limited published data on the use of CPP in human medicine, but it may be used in lieu of FFP for treatment of disseminated intravascular coagulation and for plasma exchange in cases of thrombotic thrombocytopenic purpura. In veterinary medicine, CPP is marketed for use in hypoalbuminemic patients and for treatment of vitamin K antagonist rodenticide toxicity,
although no previous published data exists. To our knowledge, this is the first published report on the use of CPP in canine patients.

Given that the pre and post albumin were significantly different and the rate of CPP administration was significantly associated with the delta albumin, one can infer that the administration of CPP helped to increase the albumin. Another potential explanation would be improvement in the patient, resulting in decreased albumin loss. Given that the infusions were administered because of concern for the critical status of the patients and the fact that many of the patients died, the increase in albumin was unlikely to be entirely based on patient factors. The pre- and post-infusion albumin were not associated with survival. The delta COP and rate of administration of CPP was not significantly associated, however the delta COP and HES rate of administration were significantly associated. This is not surprising, given that HES has a higher oncotic pressure than plasma. A previous study reported HES 6% 670/0.75/4:1 to have a COP of 32.7 mmHg, while our study found a COP of CPP to be 14.5 mmHg. Given this large difference in COP between the two products, whatever COP benefit may have been gained by increasing albumin was likely overshadowed by the COP influence of HES.

The SAFE study compared normal saline and 4% albumin solution, a similar concentration as the CPP used in our study, for fluid therapy in intensive care unit patients. There was increased survival in the patients receiving 4% albumin in the septic subgroup in this study. The baseline albumin was 25 g/L (±0.73), which was higher than the pre-infusion albumin in our study. The dose of 4% albumin administered on day 1 was 0.76 g/kg (±0.62), compared to our dose of CPP 1.37 g/kg (±0.46), assuming that the CPP transfused in our study had an albumin content of 31.7 g/L as per the findings in Chapter 2. The ALBIOS study also found increased survival in septic
shock patients receiving 20% albumin in combination with crystalloids, compared to those receiving only crystalloids.\textsuperscript{13} In that study, the baseline albumin was $24 \text{ g/L (±0.6)}$, which was also higher than in our study, with a target albumin of $>30 \text{ g/L.}\textsuperscript{13}$ The daily doses used were $0.857 \text{ g/kg}$ if the albumin was $< 25 \text{ g/L}$ and $0.571 \text{ g/kg}$ if the albumin was 25-30 g/L, which was lower than our dose.\textsuperscript{13}

In our study, non-survivors were more likely to be older, which is not surprising given the potential lack of physiologic reserves and increased likelihood of concurrent disease in older patients. Interestingly, the SPI2 score was not found to be associated with survival. The SPI2 score has been previously reported to predict 30 day survival for a heterogenous intensive care unit canine patients, but seems to not be able to adequately predict survival in our specific patient population.\textsuperscript{59} No other variables were associated with survival.

The retrospective nature of this study was an obvious limitation, as we were unable to ensure consistency of treatment or timing of sampling of albumin or COP. This study included a variety of disease processes and patient characteristics, which made interpretation of the results more difficult, given that some patients had on-going and irreversible loss of albumin (such as the patient with severe protein losing enteropathy), while others needed albumin and oncotic support only during a short period of time while an underlying disease process was treated (such as parvoviral infection). The small number of patients was another limitation and did not allow adequate statistical evaluation given the wide variety in disease processes and concurrent crystalloid therapy. The total protein of the fluid obtained from Jackson-Pratt drains and the urine output was not consistently documented in the medical records and therefore was not evaluated. Knowing this information would have allowed calculation of patient fluid balance and monitoring
of loss of proteinaceous fluid. As all of the dogs in our study received CPP, it cannot be ruled out that the patients’ albumin and/or COP would have improved without the use of CPP. Additionally, this study did not provide direct comparison to patients receiving either no plasma products or FFP, however given our previous evaluation of the albumin content of CPP, one would expect the delta albumin and COP to be similar between patients receiving either FFP or CPP. It should be noted that fluid therapy in critically ill patients is complex and requires constant evaluation and adjustment based on the dynamic needs of the patient. There is no single ideal fluid for a critically ill patient and a combination therapy of crystalloids and colloids, balancing the risks and benefits of each product, is recommended. Most of the patients received concurrent HES during the CPP infusion that could have impacted their COP, however this would not explain the increases observed in albumin.

This study found that the albumin increased after CPP continuous rate infusion in critically ill canine patients, suggesting that it may be a viable option for treatment of hypoalbuminemia. Further clinical studies are needed to evaluate the potential use of CPP for albumin and oncotic support.
Footnotes

a. Zephiran Chloride, Lab Stores, Columbus, Ohio and Dermachlor Solution (2% chlorhexidine gluconate), Henry Schein, Dublin, OH.

b. Anticoagulant citrate phosphate dextrose solution, USP (CPD) BLOOD-PACK unit; transfer-pack container with ADSOL red cell preservation solution, quadruple set, Fenwal, Lake Zurich, IL.

c. Sorvall RC3B plus centrifuge, Thermo Fisher Scientific, Waltham, MA.

d. Terumo SCD 312 sterile tubing welder, Terumo BCT, Lakewood, CO.

e. Microcentrifuge tubes, Fisherbrand, Waltham, MA.

f. Freezer model UPF3030A18, Kendro Laboratory Products, Asheville, NC.

g. Satellite bags without anticoagulant, steriley removed from the quadruple set\textsuperscript{ii}, Fenwal, Lake Zurich, IL.

h. Roche Cobas c501, Roche Diagnostics, Basel, Switzerland.

i. Diagnostica Stago, Parsippany, NJ.

j. 4420 Colloid Osmometer, Wescor, Logan, UT.

k. MedCalc Statistical Software version 15.4 (MedCalc Software bvba, Ostend, Belgium; 2015)
References


44. Pet Blood Bank UK. Canine Cryo-Supernatant Product Data Sheet.


Appendix A: SPI2 score

SPI2 score\textsuperscript{59}:

\[
\text{Logit } P = 0.3273 + (0.0108 \times \text{MAP (mmHg)}) - (0.0102 \times \text{respiratory rate (bpm)}) - (0.2183 \times \text{creatinine (mg/dL)}) + (0.0164 \times \text{PCV (\%}) + (0.3553 \times \text{albumin (g/dL)}) - (0.1184 \times \text{age (years)}) - (0.8069 \times \text{medical vs. surgical (medical = 1, surgical = 0)})
\]

Logit P is then solved for P using $P = \frac{e^{\text{logit } P}}{1 + e^{\text{logit } P}}$