

# Evaluation of the effect of fresh-frozen plasma transfusion on circulating hyaluronic acid concentration in critically ill dogs: a pilot study

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## OBJECTIVE

To describe changes in circulating hyaluronic acid (HA) concentration, a biomarker of endothelial glycocalyx degradation, after administration of fresh-frozen plasma (FFP) in critically ill dogs.

## ANIMALS

12 client-owned dogs receiving an FFP transfusion due to underlying disease.

## METHODS

Plasma samples were collected for HA concentration measurement pre-FFP transfusion (T0) and 10 minutes (T10) and 90 minutes (T90) following completion of FFP transfusion of a minimum volume of 7 mL/kg. Hyaluronic acid was also measured in the transfused FFP units following in-house validation of a commercial HA assay on citrate phosphate dextrose-anticoagulated plasma. Potential associations of the difference between pre-FFP and post-FFP HA plasma concentrations with the volume of FFP transfused, the cumulative volume of IV fluids administered during the study period, and the HA concentration in the transfused unit were explored.

## RESULTS

Concentrations of HA were not significantly different between pre- and post-FFP transfusion measurements. The volume of FFP transfused, the cumulative volume of other IV fluids administered during the study time, and the concentration of HA in the FFP units had no significant effect on the change in HA concentration following FFP transfusion in this study.

## CLINICAL RELEVANCE

This pilot study did not demonstrate an association between FFP administration and changes in plasma HA concentration. The results of this study may serve to help design future research. A commercial assay was validated to measure HA in citrate phosphate dextrose-anticoagulated plasma.

**Keywords:** fresh-frozen plasma, endothelial glycocalyx, transfusion, canine, hyaluronan

The endothelial glycocalyx (EG), a mesh-like structure covering the inner surface of blood vessels, has a key role in maintaining homeostasis in the body.<sup>1,2</sup> Its dynamic structure of assembled membrane-bound proteoglycans, glycosaminoglycans, glycoproteins, and plasma proteins is crucial in regulating transvascular fluid flux, inflammation, hemostasis, and vascular tone.<sup>1,3,4</sup> Degradation of the EG is thought to significantly contribute to the

progression of organ dysfunction in conditions such as sepsis and trauma.<sup>1,5-9</sup> Although data on the EG are still limited in veterinary medicine, EG degradation has been documented in dogs with sepsis,<sup>10-12</sup> hemorrhagic shock,<sup>13</sup> and ischemia-reperfusion injury due to cardiopulmonary bypass.<sup>14</sup> Components of the EG shed into circulation after its degradation can be used as biomarkers of endothelial injury and have shown reliable and timely correlation with other structural and functional indicators of EG degradation in experimental models and humans.<sup>15-18</sup> Human studies<sup>15,16,19</sup> have demonstrated that patients with higher concentrations of EG biomarkers on presentation or an increase in concentration during the

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first days of hospitalization have an increased risk of organ dysfunction and death, indicating that these biomarkers can be valuable prognostic indicators.

Although IV fluid therapy is lifesaving, over the last decade, the potential adverse effects of liberal IV fluids have been increasingly recognized, especially in patients with sepsis and trauma.<sup>20-23</sup> Liberal fluid therapy and fluid overload are implicated in EG degradation, and cumulative EG damage can contribute to negative outcomes, as it can lead to interstitial edema, hypoperfusion, coagulopathy, and dysregulated systemic inflammation.<sup>24-26</sup> This is particularly relevant as patients requiring fluid resuscitation for hemodynamic stabilization, such as patients with sepsis and hemorrhage, are often already at risk of EG degradation. Recently, experts have recommended more conservative fluid delivery in human and veterinary medicine; however, evidence-based recommendations remain lacking.<sup>27-30</sup> Fluid strategies that both optimize macrohemodynamic parameters and support the microcirculation are needed.

Plasma, the liquid component of blood, contains numerous biologically important proteins. Fresh-frozen plasma (FFP) is defined as plasma collected from healthy donors, frozen within 8 hours of collection, and stored at less than  $-20^{\circ}\text{C}$  for less than 1 year.<sup>31</sup> The predominant indication for FFP transfusion is for the treatment of coagulopathies.<sup>32</sup> Its use has however become more frequent in various clinical scenarios, including as part of fluid resuscitation for patients with trauma<sup>33,34</sup> and sepsis.<sup>32,35</sup> Administration of blood products in a 1:1:1 ratio of packed RBCs (pRBCs), plasma, and platelets is recommended in human medicine for resuscitation of hemorrhaging trauma patients, based on evidence of clinical benefit.<sup>33,34</sup> Administration of FFP in sepsis can be considered for volume replacement and oncotic support, as well as theoretically beneficial anti-inflammatory properties of antithrombin, protein C, and protein S; however, this remains much more controversial both in veterinary patients and humans.<sup>31,32,36,37</sup> In veterinary patients, reported indications for FFP transfusion historically include coagulopathy with or without clinical bleeding, albumin support, and provision of immunoglobulins or  $\alpha$ -macroglobulins.<sup>31,38,39</sup> However, the use of FFP for volume resuscitation in veterinary patients with hemorrhage despite the lack of documented coagulopathy, and for oncotic support as part of fluid resuscitation for septic shock, has also been increasingly reported.<sup>40,41</sup> Beyond providing coagulation factors and a limited amount of albumin, experimental studies<sup>42-45</sup> and human clinical trials have shown that FFP can have a protective and restorative effect on the EG. A decrease in circulating biomarkers of EG degradation and an increase in EG thickness have been documented within 1 hour of FFP transfusion in these studies.<sup>42-45</sup> The mechanisms behind the benefit of FFP on the EG are still not fully elucidated. Proteins such as sphingosine-1-phosphate, adiponectin, or fibrinogen could be key players in stopping the EG degradation process, possibly via

inhibition of the enzymatic destruction of its components.<sup>46-49</sup> The modulation of gene expression of EG components has also been proposed as a mechanism of restoration, although this effect would be more delayed.<sup>50</sup>

The potential benefits of FFP on the EG have not been studied in veterinary medicine, and initial data are needed to evaluate this promising EG-protective blood product. Should its benefits be confirmed in clinical research, FFP could be recommended as part of volume resuscitation and mitigate EG degradation in patients at risk. Hyaluronic acid (HA) is a glycosaminoglycan that has been validated as a biomarker of EG degradation in dogs.<sup>12</sup> Its concentration has been shown to change in a timely manner with EG degradation from shock followed by resuscitation.<sup>13</sup> An increase in HA concentration has been found to be correlated with decreased EG thickness and increased concentration of syndecan-1, another EG biomarker commonly used in human medicine.<sup>51</sup> Resolution of EG degradation is expected to result in resolved HA shedding, leading to a quick decrease in HA plasma concentration based on its short half-life in circulation (2 to 5 minutes).<sup>52</sup> Quantification of HA concentrations in canine blood using commercial ELISA kits has been previously validated and used in research.<sup>10,12,13,53-57</sup> The goal of this pilot study was to observe the degree of change in HA concentration in critically ill dogs following FFP transfusion and gather data to help determine the required sample size, appropriate sampling time points, and relevant additional factors to investigate in future studies. This was an observational study. Plasma HA concentrations were measured pre- and post-FFP transfusion in dogs, and the effects of the volume of FFP transfused, the volume of other IV fluids administered during the study time, and the HA concentration in the FFP transfused were described. The study hypothesized that if FFP reduces EG shedding, HA concentrations would decrease within a few minutes of FFP administration.

## Methods

### Study design

This was a prospective observational study conducted in the ICU of a veterinary teaching hospital between November 2022 and September 2023. All procedures were approved by the Institutional Animal Care Committee (AUP No. 4950), and informed owner consent was obtained for all dogs.

Dogs were enrolled if they were admitted to the ICU and received an FFP transfusion at the clinician's discretion for any indication. Dogs had to receive a minimal volume of 7 mL/kg of FFP to be included. Dogs receiving plasma products not meeting the definition of FFP were not included. There was no specific requirement for transfusion duration, and a transfusion was defined as an uninterrupted administration of FFP (from single or multiple FFP units). Dogs were excluded from the study if they weighed less than 5 kg (to avoid iatrogenic anemia), if they

received any portion of the FFP transfusion during general anesthesia or surgery, if they received FFP as part of therapeutic plasma exchange, or if the collection of posttransfusion blood samples was not possible. Dogs with a history of comorbidities were not excluded; however, this information was recorded. All dogs received standard-of-care therapy at the clinician's discretion.

## Clinical data collection

Patient signalment and weight were recorded, as well as known comorbidities that might affect HA concentration, including liver disease, diabetes mellitus, malignancy, renal disease, osteoarthritis, and ischemia-reperfusion from cardiac arrest and return of spontaneous circulation. Data recorded at admission included clinical signs and laboratory findings from blood gas analysis, hematology, and biochemistry profiles. The illness severity score at the time of admission was determined using the acute patient physiological and laboratory evaluation fast (APPLE<sub>fast</sub>) score.<sup>58</sup> The incidence of hyperglycemia during the study period was also recorded. The primary diagnosis and indication for FFP transfusion were recorded. The indication for FFP transfusion was classified as coagulopathy (without hemorrhagic shock), hemorrhagic shock, and hypovolemic shock with hypoproteinemia less than 5.0 g/dL (not due to hemorrhage).

The volume of FFP administered, duration of the transfusion, and age of the transfused FFP were recorded. The type and volume of all other IV fluids (crystalloids and blood products) administered during the study time were also recorded. Medications administered during the study time were at the clinician's discretion. The outcome (survival to discharge, humane euthanasia, or death) was also recorded. For euthanized patients, the reason for the decision was characterized as financial or due to a suspected grave prognosis.

Fresh-frozen plasma was obtained from the hospital's blood bank. It was collected from healthy screened donors,<sup>59</sup> frozen within 8 hours of collection, and stored at  $-20^{\circ}\text{C}$  for less than 365 days. Plasma units were thawed just before transfusion, and each unit was administered over less than 6 hours. Patients received a test dose at the beginning of each transfusion and were closely monitored during the transfusion. Following our transfusion protocol, other medications were withheld during the transfusion as much as possible.

## Sample collection and biomarker measurement

Blood (3 mL) was collected from each dog at the following predefined time points: within 1 hour before starting the transfusion (T0), at 10 minutes posttransfusion (T10), and at 90 minutes posttransfusion (T90). A sample (1 mL) was also collected from the FFP unit (if more than 1 FFP unit was transfused, the FFP samples were pooled for a total volume of 1 mL). All samples were processed in

preparation for HA measurement following the manufacturer's instructions. Blood samples were collected via jugular or saphenous venipuncture or via an indwelling sampling catheter if available (using a routine blood discard protocol of at least 1 mL before blood collection to prevent contamination with heparin or dilution with saline). Blood was placed in a vacuum-sealed plastic tube with EDTA anticoagulant and was centrifuged at  $1,000 \times g$  for 15 minutes within 30 minutes of collection. The plasma was then retrieved, aliquoted, and stored at  $-80^{\circ}\text{C}$  for later batch analysis. The FFP units were all anticoagulated with citrate phosphate dextrose (CPD). The samples from FFP units were collected into vacuum-sealed plastic tubes without additives. Samples were aliquoted and stored at  $-80^{\circ}\text{C}$ .

Biomarker measurement was conducted at a different site (Ohio State University). All samples were transported via overnight courier on dry ice. Upon arrival, the frozen samples were transferred into a  $-80^{\circ}\text{C}$  freezer until later batch analysis. The samples were only thawed at the time of analysis. Biomarker measurement was performed using a commercial ELISA kit previously validated for use in dogs (Quantikine ELISA Hyaluronan Immunoassay; R&D Systems).<sup>10,12,13,53-57</sup> The measurements were performed in duplicate and following the manufacturer's instructions. The assay's lower limit of detection reported by the manufacturer was 0.068 ng/mL.

In the absence of previous validation of HA measurement using this ELISA kit with canine CPD-anticoagulated blood, a partial validation study was conducted. Plasma anticoagulated with CPD was collected from 5 healthy blood donors at the time of blood collection and from 5 sick dogs. Sick dogs were client-owned dogs who were hospitalized for sepsis secondary to arthritis ( $n = 1$ ), severe pancreatitis (2), and severe pneumonia (2). Whole blood samples were collected from the dogs into CPD-anticoagulated plastic tubes at a standard CPD-to-blood ratio of 1 to 7. Hyaluronic acid was measured in duplicate in each of these samples. The mean  $\pm$  SD concentration of HA in CPD plasma from healthy dogs was  $40.3 \pm 16.6$  ng/mL, and the range was 24.1 to 66.5 ng/mL. For sick dogs, the mean  $\pm$  SD concentration of HA was  $158.2$  ng/mL ( $\pm 99.3$ ), and the range was 69.1 to 305.9 ng/mL. Intra-assay variability was determined from 10 duplicate measurements which yielded a coefficient of variation of 5.47%. Linearity was evaluated by serial dilutions of baseline and spiked samples from 4 healthy dogs and 2 sick dogs to obtain 50%, 25%, 12.5%, and 6.25% dilutions. Dilutional linearity was plotted as the observed-to-expected ratio calculated from the baseline and dilution factor. Linear regression with the associated coefficient of correlation  $R^2$  was determined (**Supplementary Figure S1**). Linearity was excellent for dilution of samples from healthy dogs ( $R^2$  values were all above 0.98, with an average of 0.99) and acceptable for dilution of samples from sick dogs ( $R^2$  of 0.88 and 0.91). Values from another validation study performed by Devriendt et al<sup>57</sup> using healthy

dog serum (using the same ELISA assay) were used to perform an unpaired *t* test with our healthy dog samples. No statistically significant differences were identified between the 2 sets of values ( $P = .92$ ).

## Statistical methods

Data were analyzed for normality by examination of the residuals, quantile-quantile plots, and normality tests that included the Shapiro-Wilk test, Kolmogorov-Smirnov test, Cramer-von Mises test, and Anderson-Darling test. Results are presented as means  $\pm$  SD when normally distributed or median  $\pm$  interquartile range (IQR) when not normally distributed. The concentration of HA was compared between each time point using a *t* test. To investigate possible predictors of the HA concentration difference over time, a general linear model for repeated measures was used. The model included as possible explanatory variables the volume of FFP administered, the volume of other IV fluids administered, and the HA concentration in the FFP unit as well as interaction terms. Nonsignificant effects were removed when  $P > .05$ , and the model was simplified. Several correlation structures were tested to account for within-subject correlation. A first-order autoregressive correlation structure had the best fit based on the Akaike information criterion. If the overall *F* test was significant, post hoc pairwise comparisons were done based on a *t* test. Statistical analysis was done using commercial software (SAS/STAT 9.4; SAS Institute).

**Table 1**—Indications for fresh-frozen plasma (FFP) transfusion in dogs included in the study.

Indication for FFP transfusion/etiology	No. of dogs	
Hemorrhagic shock		
Trauma	2	5
Gastrointestinal bleeding	2	
Surgical bleeding	1	
Hypovolemic shock with hypoproteinemia		
Sepsis	4	5
Liver failure	1	
Coagulopathy		
Anticoagulant rodenticide	1	2
DIC secondary to diffuse neoplasia	1	

DIC = Disseminated intravascular coagulation.

**Table 2**—IV fluid administration in dogs during the study time (fresh-frozen plasma [FFP] excluded).

Type of IV fluids	No. of dogs who received fluids	Mean volume administered between T0 and T10 (n = 12)	Mean volume administered between T10 and T90 (n = 9)	Mean volume administered between T0 and T90 (n = 9)
Isotonic crystalloids (mL/kg)/(mL/kg/h)	11	4.4 $\pm$ 3.9/1.5 $\pm$ 1.7	3.9 $\pm$ 2.3/3.0 $\pm$ 1.8	8.7 $\pm$ 5.6/1.8 $\pm$ 1.4
Hypertonic saline (mL/kg)	1	5.0 $\pm$ 0	0 $\pm$ 0	5.0 $\pm$ 0
Packed RBCs (mL/kg)	3	7.8 $\pm$ 2.5	0 $\pm$ 0	7.8 $\pm$ 2.5
Total cumulative (mL/kg)	12	6.8 $\pm$ 5.7	3.9 $\pm$ 2.3	10.7 $\pm$ 7.5

Results are presented as the number of dogs or means  $\pm$  SD. Isotonic crystalloids were sometimes delivered during the FFP transfusion, and volumes are presented as both mL/kg and mL/kg/h to account for variable durations of FFP transfusions (variable time between baseline [pretransfusion; T0] and 10 minutes post-FFP transfusion [T10]). Hypertonic saline was administered as a bolus and packed RBCs were administered over a variable time.

T90 = 90 minutes post-FFP transfusion.

## Results

### Animal characteristics

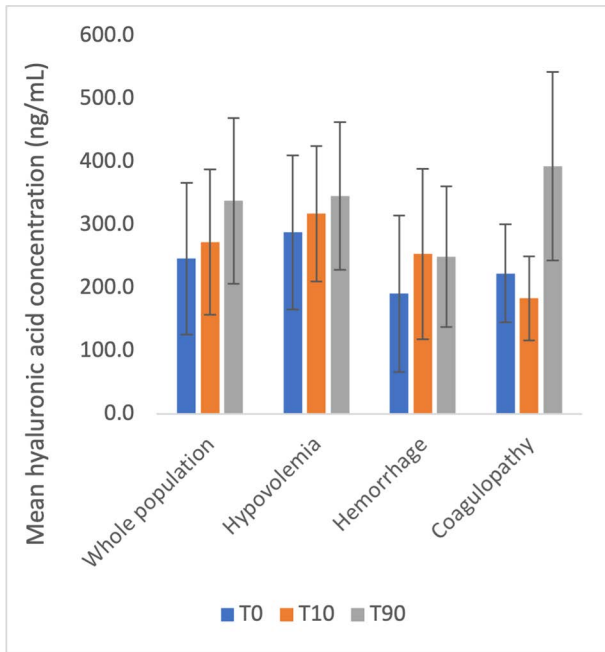
Thirteen dogs were enrolled in the study. One dog was later excluded due to cardiac arrest during the FFP transfusion, leaving 12 dogs for study analysis. Three dogs (3/12) did not have a T90 time point data because they were undergoing surgery at that time ( $n = 2$ ) or because another plasma transfusion had been started between the T10 and T90 time points, and therefore collection of a 90 min posttransfusion sample was not possible (1). Most dogs were mixed-breed dogs (5), and among pure-breed dogs, there were 3 Labrador Retrievers and 1 of each of the following breeds: Shih Tzu, Great Dane, Schnauzer, and Golden Retriever. The mean  $\pm$  SD age was 7.3  $\pm$  4.0 years, and the mean  $\pm$  SD weight was 29.3  $\pm$  15.1 kg. There were 5 neutered males, 6 spayed females, and 1 intact male. Comorbidities included malignancy ( $n = 3$ ), diabetes mellitus (1), and cardiopulmonary arrest with successful return of spontaneous circulation 8 hours before study inclusion (1). Four dogs had hyperglycemia during the study. Blood glucose was stable throughout the study time for 3/4 dogs, and 1 dog had a single episode of mild hyperglycemia (9 mmol/L) at 90 minutes following the FFP transfusion.

The illness severity APPLE<sub>fast</sub> score was calculated for 11/12 dogs, while 1 dog had insufficient data available for the calculation. The mean  $\pm$  SD APPLE<sub>fast</sub> score was 30.8  $\pm$  3.7. Septic shock was diagnosed in 4 dogs based on the need for vasopressors to maintain mean arterial pressure greater than or equal to 65 mmHg.

The indications for FFP transfusion were hemorrhagic shock, hypovolemic shock with hypoproteinemia, and coagulopathy of different underlying etiologies (**Table 1**). The dogs with coagulopathy had evidence of hemorrhage (eg, hemothorax) but were hemodynamically stable.

### Plasma and IV fluid administration

Before study inclusion, isotonic crystalloids (Plasmalyte-A; Baxter) were being delivered to 10/12 dogs. The volume and duration of these fluids administered in the prestudy time frame were not further characterized. One dog had received an FFP transfusion (7 mL/kg) 14 hours before inclusion



**Figure 1**—Mean plasma hyaluronic acid concentration at each time point for each indication of fresh-frozen plasma (FFP) transfusion. Indications for FFP transfusion are shown as hypovolemia (hypovolemia with hypoproteinemia), hemorrhage (hemorrhagic shock), and coagulopathy. The error bars represent SD. There was no statistically significant difference between the concentrations at each time point. T0 = Baseline (pre-FFP transfusion). T10 = 10 minutes post-FFP transfusion. T90 = 90 minutes post-FFP transfusion.

in the study, and 1 dog had received a stored whole blood transfusion (17 mL/kg) 6 hours prior. The other dogs did not receive plasma products in the 7 days before their inclusion in the study.

The median ± IQR volume of FFP transfused was 9.3 ± 2.2 mL/kg, and the median ± IQR duration of the FFP transfusion was 240 ± 49 minutes. The age of the FFP units ranged from 7 to 362 days.

Other IV fluids delivered during the study period included isotonic crystalloids (Plasmalyte-A; Baxter), hypertonic saline (5% sodium chloride; B Braun Ltd), and pRBC transfusions (Table 2).

## Hyaluronic acid

There was no statistically significant difference between HA concentrations at T0 and T10 ( $P = .6585$ ), between T0 and T90 ( $P = .3410$ ), and between T10 and T90 ( $P = .1415$ ) (Figure 1; Table 3). Individual concentrations of HA are presented (Supplementary Figure S2). The mean ± SD HA concentration in the FFP units was 57.6 ± 29.0 ng/mL. There was no statistically significant effect of the volume of FFP transfused ( $P = .82$ ), the cumulative volume of other IV fluids ( $P = .28$ ), or the HA concentration in the FFP unit ( $P = .65$ ) on the difference in HA concentration between pre-FFP and post-FFP time points.

## Outcome

Five dogs (42%) survived to discharge, 3 dogs (25%) died, and 4 dogs (33%) were euthanized (all due to poor prognosis, none for financial reasons). No transfusion reactions were observed during the study.

## Discussion

This study explores the effects of FFP transfusion on plasma HA concentration in critically ill dogs as a biomarker for EG degradation. Of note, an increase in circulating HA reflects active EG degradation, and the study hypothesized that halting of this destruction should demonstrate a decrease in circulating HA levels. No significant effect of FFP on HA concentration was observed in the present study over 90 minutes following transfusion. Despite a lack of HA change in this study, the benefit of FFP on the EG has been demonstrated in several murine models of hemorrhagic shock with reduction in EG biomarkers, reduction in vascular permeability, and increase in EG thickness following FFP transfusion.<sup>5,44,45,60–62</sup> However, 1 study<sup>63</sup> conducted in a murine model of sepsis showed reduced vascular permeability after FFP resuscitation despite no effect on endothelial biomarkers including syndecan-1, thrombomodulin, von Willebrand factor, and intercellular adhesion molecule 1. The potential benefit of FFP on the EG in disease processes other than hemorrhagic shock is still highly uncertain. In a human study,<sup>64</sup> FFP transfusion led to decreased concentrations of syndecan-1, an EG biomarker. In that study, 45% of

**Table 3**—Mean hyaluronic acid concentrations at each time point and differences in concentrations between pre- and post-fresh-frozen plasma (FFP) time points for each group of FFP transfusion indication.

Indication for FFP	HA concentration (ng/mL)			Difference between T10 and T0	Difference between T90 and T0
	T0	T10	T90		
Hypovolemia with hypoproteinemia	306.7 ± 136.8 (n = 5)	340.0 ± 98.3 (n = 5)	381.6 ± 45.0 (n = 4)	33.3 ± 62.3 (n = 5)	51.0 ± 100.5 (n = 4)
Hemorrhagic shock	207.5 ± 166.8 (n = 5)	254.8 ± 172.0 (n = 5)	249.1 ± 179.6 (n = 3)	47.3 ± 132.0 (n = 5)	30.4 ± 132.6 (n = 3)
Coagulopathy	250.5 ± 56.2 (n = 2)	207.6 ± 36.3 (n = 2)	404.6 ± 320.6 (n = 2)	-42.9 ± 19.9 (n = 2)	154.1 ± 264.4 (n = 2)
Whole population	239.8 ± 123.3 (n = 12)	269.3 ± 119.2 (n = 12)	340.8 ± 119.2 (n = 9)	29.5 ± 95.0 (n = 12)	92.5 ± 119.4 (n = 9)

Results are presented as means ± SD. There was no statistically significant difference between the concentrations at each time point. T0 = Baseline (pre-FFP transfusion). T10 = 10 minutes post-PP transfusion. T90 = 90 minutes post-FFP transfusion.

the patients had sepsis and none had hemorrhage, suggesting a benefit of FFP on the EG in sepsis; however, the study sample was small (33 patients), and the benefits of FFP were not specifically investigated for each underlying condition. Clinical studies exploring the effects of FFP on the EG are also lacking in veterinary medicine. The only veterinary study investigating the effect of plasma-containing blood products on the EG was conducted by Smart et al<sup>13</sup> on healthy Greyhounds subjected to experimental hemorrhagic shock. Dogs resuscitated with whole blood showed a smaller increase in HA than dogs resuscitated with crystalloids; however, the effect of plasma was not specifically investigated, and several factors could have contributed to this result, including the administration of substantially higher resuscitative volumes of crystalloids (80 mL/kg) than whole blood (20 mL/kg).

The present study collected samples for HA measurement pretransfusion, 10 minutes posttransfusion, and 90 minutes posttransfusion to assess the short-term effects of FFP on the EG. A previous study<sup>64</sup> in critically ill human patients with coagulopathy showed a decrease in the EG biomarker syndecan-1 within 10 minutes after FFP transfusion, although no other measurements were performed beyond 10 minutes. In experimental murine models showing a benefit of FFP on the EG following hemorrhagic shock, EG biomarkers (syndecan-1 and heparan sulfate) and EG thickness were typically measured within 1 hour following resuscitation with FFP.<sup>60-62</sup> In a murine model of pneumosepsis, syndecan-1 was measured only 4 hours after the end of resuscitation and was not different in concentration between rats resuscitated with crystalloids and rats resuscitated with FFP.<sup>63</sup> That study<sup>63</sup> was limited to a sepsis model, unlike the other experimental hemorrhagic shock models, and used limited resuscitation volumes compared to the other studies. Regardless, the duration of potential effects on the EG of transfused FFP is unknown at this time, and there are insufficient data to support an effect beyond one hour. Although HA has not been the biomarker used in previous studies of FFP transfusion on the EG, a rapid decrease in HA concentration (within 10 minutes) would be expected after resolution of EG degradation based on its short half-life,<sup>52</sup> similar to other previously investigated biomarkers. Moreover, a 90-minute sampling time point was investigated to assess the potential change in HA over time following FFP transfusion. No effect was recognized. An effect of FFP on HA was not explored beyond 90 minutes due to the risk of HA being affected by the progression of disease and ongoing clinical needs of the patient, including IV fluids, therapeutic interventions, and surgical procedures.

The volume of other IV fluids did not have a significant effect on the difference between pre- and posttransfusion HA concentrations in this study. However, administration of IV crystalloids has been associated with EG degradation in humans<sup>24,26,65,66</sup> and dogs.<sup>10,13,56</sup> Concurrent crystalloid administration to dogs during the study period could therefore

interfere with the effects of FFP on HA concentrations, despite the lack of demonstrated effect in our small sample size. Red blood cell transfusion seems to have little effect on the EG,<sup>42,67</sup> although data on this topic are very limited. The cumulative volume of IV fluids other than FFP administered over the study time should therefore be considered as a confounding factor for changes in HA concentration. Moreover, most of the dogs in our study (n = 10) were hospitalized and receiving IV crystalloids before enrolment. In previous experimental studies,<sup>44,60-62</sup> animals were resuscitated with FFP immediately after shock, without receiving prior IV fluids. The benefit of FFP administered after initial crystalloid resuscitation is unknown. In human trauma patients with massive hemorrhage, early resuscitation with pRBC, FFP, and platelets is recommended rather than using large volumes of crystalloids, and plasma resuscitation should be used within 2.5 hours of admission.<sup>33,68</sup> The ideal timing to transition from crystalloid to FFP resuscitation has not been determined in veterinary patients and likely depends on patient status and underlying disease.

Since this study was observational, the volume of FFP transfused was not standardized. There are very limited data on the FFP volume needed to achieve a potential benefit on the EG. The median of 9.3 mL/kg of FFP transfused during this study may have been insufficient to demonstrate an effect of FFP, and the sample size was likely too small to demonstrate an influence of the FFP volume transfused on HA changes. We elected to include dogs receiving at least 7 mL/kg of FFP because this represented an average FFP unit (about 250 mL) for a 35-kg dog. Previous investigations<sup>60-64</sup> have explored FFP volumes ranging from 8 to 15 mL/kg. In the murine models showing a benefit of FFP transfusion on the EG for resuscitation of hemorrhagic shock, FFP was administered at 12 to 15 mL/kg of FFP, which in these studies<sup>60-62</sup> was close to the volume of shed blood. Similarly, in the study<sup>64</sup> on critically ill coagulopathic human patients showing improved syndecan-1 after FFP transfusion, 12 mL/kg of FFP was administered. Conversely, in the murine experimental model of sepsis previously described, only 8 mL/kg of FFP was administered, and as previously mentioned the endothelial biomarkers studied were not improved following FFP transfusion compared to crystalloids.<sup>63</sup> Considering the large range of canine body weights and different FFP needs based on the underlying disease, standardization of FFP volume in a clinical study might be difficult to achieve.

In the study herein, the time over which FFP transfusions were administered was also not standardized. In previous experimental studies,<sup>13,60-63,69</sup> FFP was administered as a resuscitation fluid over 1 hour or less; however, the duration of FFP transfusions in patients can vary depending on indication and patient status. The effect of transfusion duration on the possible benefits of FFP on the EG has not been investigated. Storage conditions can also affect the plasma's EG-protective properties, as FFP thawed for 5 days partially loses its beneficial effects

on the EG based on a murine model of hemorrhagic shock.<sup>44</sup> All plasma delivered during this study was thawed immediately before its administration. The influence of the age of the transfused FFP on its effect on the EG was not investigated in our study due to the small sample size.

Dogs with any indication of FFP transfusion and underlying disease were included in this study. Since the mechanism of FFP benefit on the EG has not been elucidated, this benefit could vary based on the underlying cause of EG degradation (eg, hemorrhage, sepsis). It would be interesting to compare the effects of FFP in dogs with preclassified diseases: in dogs with and without evidence of systemic inflammatory response syndrome and in dogs with and without hemorrhage. The severity of the disease could also play a role in response to FFP in terms of EG degradation. Unfortunately, a correlation between HA concentration and disease severity (APPLE score) could not be investigated in this study due to the small sample size.

Several factors may have affected the lack of significant effect of FFP on HA in this study. A small sample size, the nonstandardized volume of FFP transfused, the duration of transfusion, and additional administration of crystalloids could have contributed to a lack of noticeable benefit of FFP on the EG, as measured by HA. However, the HA concentration difference between pre- and post-FFP samples was very variable between dogs, and an increase in HA concentration was even noted for several patients (up to 2-fold). This was unexpected based on our hypothesis. The patient population included critically ill dogs and their disease process remained ongoing during the study period, with active hemorrhage, disseminated intravascular coagulation, and sepsis. This differs from previous experimental studies<sup>5,13,44,45,60-62</sup> where hypovolemic shock was induced (eg, blood withdrawal) and terminated at the time of resuscitation. In sick patients, it is possible that active EG degradation exceeds the positive effects of the FFP delivered, with an associated variable increase in HA concentration.

The FFP units from healthy donors contain HA and the concentration of HA within these units could influence the transfused dogs' posttransfusion HA concentrations. Our study included a partial in-house validation of a commercial ELISA kit (Quantikine ELISA Hyaluronan Immunoassay; R&D Systems) for the measurement of HA concentration in canine CPD plasma. This validation process confirmed that the ELISA kit was reliably measuring HA using CPD-anticoagulated plasma. Concentrations of HA measured in our healthy dogs' CPD plasma were not significantly different from the ones measured in another validation study using serum.<sup>57</sup> Our median HA concentration was also similar to the ones reported in previous studies including healthy dogs and using the same ELISA kit, although no statistical comparison could be performed with these studies (**Supplementary Table S1**).<sup>12,53-57</sup> Based on these results, intra-assay variability, and linearity, the measurement of HA concentration in the FFP units

(CPD plasma from healthy blood donors) using this commercial ELISA kit was deemed reliable.

No statistically significant effect of the FFP HA concentration was demonstrated in our study. Considering that HA concentrations in the FFP units were substantially lower than most of the patients' pre- and post-FFP HA concentrations, the FFP HA was unlikely to significantly influence patients' HA concentrations. A dilutional effect based on the volume of FFP administered would be more likely. A correlation between the age of FFP units and their HA concentration could not be demonstrated due to small sample size.

This study has several limitations. As discussed previously, it is a pilot study and the sample size is therefore small. There was no standardization for the administration of FFP and other IV fluids. Several dogs had comorbidities such as liver disease, diabetes mellitus, and neoplasia, which have been associated with an increase in HA concentrations.<sup>70-72</sup> Hyperglycemia has also been demonstrated to cause EG degradation in as little as 6 hours in nondiabetic human patients.<sup>73</sup> Again, in the study herein, dogs were used as their own controls, making comorbidities that affect HA unlikely to have affected results. Furthermore, although the administration of medications was kept to a minimum during transfusion, some patients received medications during the study time, which could have influenced the results. Very little is known about the effect of medications on HA concentration. Moreover, HA was the only biomarker measured in our study. Previously cited experimental studies<sup>5,60-62,64</sup> investigating the effect of FFP on the EG measured the EG biomarkers syndecan-1 and heparan sulfate and reported changes in their concentrations. The effect of FFP on HA might be different, as it is the only glycosaminoglycan anchored to the endothelial cell membrane via the protein CD44.<sup>1</sup> Unfortunately, validated canine assays for measurement of syndecan-1 and heparan sulfate are lacking. Robust validation of HA assays in dogs prompted its use in this study as a marker for EG degradation. Ideally, a combination of biomarkers to assess the EG response to FFP is indicated. The use of biomarkers of EG degradation also only provides information on the severity of EG degradation and does not allow investigations on EG restoration, which would require visualization of EG thickness, endothelial immunostaining of EG components, or measurement of endothelial permeability.

In summary, FFP has shown promising effects on the EG in experimental and limited human clinical studies.<sup>5,44,60-64</sup> However, using HA as a sole biomarker of EG degradation, our study did not confirm the hypothesis that HA concentrations would decrease following FFP administration in ill dogs. The utility of incorporating FFP in the management of critically ill dogs to affect the EG requires further assessment. Fresh-frozen plasma could be indicated as a resuscitative and/or maintenance fluid in patients at high risk of EG degradation, but FFP transfusion is not without risks and transfusion reactions are reported in 4% to 14% of dogs receiving

plasma.<sup>40,41</sup> The cost of FFP is also much higher than crystalloids. Therefore, FFP administration cannot be recommended without justified indication, and more information is needed regarding its possible benefit to the EG. This pilot study provides data to help design future research investigating the effects of FFP on the canine EG. Future studies would also need to investigate the influence of the underlying disease, the volume of FFP transfused, and the volume of crystalloids administered on the effect of FFP on the EG. Other factors including the age of the FFP transfused, the HA concentration in the FFP transfused, and the duration of the transfusion could be studied as well. Clinical and experimental studies investigating the effects of FFP on the EG for several hours after transfusion and comparing the effect of FFP and equipotent volumes of crystalloids on the EG would also be needed.

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## Supplementary Materials

Supplementary materials are posted online at the journal website: [avmajournals.avma.org](http://avmajournals.avma.org).