



Impact of general anesthesia on rotational thromboelastometry (ROTEM) parameters and standard plasmatic coagulation tests in healthy Beagle dogs

Andrea Schwarz^{a,*}, Louise F Martin^a, Francesca Del Chicca^b, Nadja E Sigrist^c, Annette PN Kutter^a

^a Department of Clinical Diagnostics and Services, Section Anaesthesiology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

^b Department of Clinical Diagnostics and Services, Clinic for Diagnostic Imaging, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

^c Department for Small Animals, Division of Emergency and Critical Care Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

ARTICLE INFO

Keywords:

Canine
Fibrinogen
Hemodilution
Hypothermia
Thromboelastography
Viscoelastic coagulation testing

ABSTRACT

Objective: To assess the influence of general anesthesia on rotational thromboelastometry (ROTEM) and standard coagulation testing in healthy dogs

Study design: Prospective experimental study

Animals: 10 healthy Beagle dogs

Methods: Dogs were administered methadone (0.2 mg/kg) intramuscularly. Anesthesia was co-induced intravenously 30 min later with midazolam (0.1 mg/kg) and propofol to effect, and maintained with sevoflurane. Crystalloids were administered at 5 ml/kg/h. Blood was sampled by direct venipuncture before induction (T0) and 3.5 h later (T3.5) and ROTEM parameters (ExTEM, InTEM, FibTEM, ApTEM), standard plasmatic coagulation tests (prothrombin time [PT], activated partial thromboplastin time [aPTT], fibrinogen concentration), hematology, ionized calcium, triglycerides, pH, lactate and body temperature were compared over time with Students t - test or Wilcoxon matched pairs signed-rank tests.

Results: The following variables dropped significantly between T0 and T3.5: body temperature ($p < 0.0001$), hematocrit ($p < 0.0001$), platelet count ($p < 0.01$), pH ($p < 0.01$), triglycerides ($p < 0.01$), fibrinogen concentration ($p < 0.01$), ExTEM, FibTEM ($p < 0.01$) and ApTEM ($p < 0.05$) clotting times. Lactate concentration ($p < 0.01$), aPTT ($p < 0.05$) and FibTEM maximum clot firmness increased ($p < 0.05$). No changes were noted in ionized calcium, PT and InTEM values.

Conclusion and clinical relevance: General anesthesia with concurrent hemodilution and hypothermia induced significant but clinically irrelevant changes in coagulation variables measured at 37 °Celsius. Blood samples from anaesthetized animals can be used for determination of coagulation status in dogs.

Introduction

Coagulopathies are a common finding in critically ill dogs (Fry et al., 2017; Herrero et al., 2020; Kelley et al., 2015; Sigrist et al., 2017) and intraoperative determination of coagulation status may become necessary in those patients, especially if active bleeding is observed. Therefore anesthesia-related changes in the assessment of coagulation have to be taken into account when therapeutic measures are taken. Additionally, many experimental studies evaluating the coagulation status before and after a specific treatment are performed in dogs under anesthesia (Boyd et al., 2018) and knowledge of a possible influence of general anesthesia

is mandatory for the appropriate interpretation of results.

Thromboelastometry parameters in people after sevoflurane or propofol anesthesia were not different to preanesthetic measurements (Koo et al., 2014). However, the effect of intra-anesthetic assessment of coagulation parameters has never been studied in people despite the widespread use of viscoelastic tests during anesthesia for trauma-related, cardiac and transplantation surgery (Agarwal & Abdelmotieieb, 2020; Sayce, Neal & Leeper, 2020). To the authors' knowledge in dogs neither intra- nor postanesthetic measurements were compared to preanesthetic measurements.

Viscoelastic tests such as rotational thromboelastometry (ROTEM)

* Corresponding author: Department of Clinical Diagnostics and Services, Section Anaesthesiology, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 258c, Zurich, 8057 Switzerland.

E-mail address: aschwarz@vetclinics.uzh.ch (A. Schwarz).

<https://doi.org/10.1016/j.vas.2021.100223>

Received 26 May 2021; Received in revised form 24 November 2021; Accepted 2 December 2021

Available online 4 December 2021

2451-943X/© 2021 The Authors.

Published by Elsevier Ltd.

This is an open access article under the CC BY-NC-ND license

(<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

measure kinetic and tensile coagulatory properties in whole blood. ROTEM parameters provide information for both the cellular and soluble components of the hemostatic process, in particular about clot kinetics, clot strength and fibrinolysis (Kol & Borjesson, 2010).

The aim of the current study was to assess the impact of a standardized general anesthesia without surgical intervention on ROTEM and standard plasmatic coagulation parameters in healthy dogs. It was hypothesized that general anesthesia with volatile anesthetics itself has no significant influence on coagulation and therefore blood samples from anaesthetized animals can be used to assess the general coagulation status of dogs.

Material and methods

This study was approved by the local ethical committee of the Canton Zurich, Switzerland Canton Zurich, Switzerland (Nr. 114/2013).

Animals

Eleven healthy research Beagle dogs undergoing a magnetic resonance imaging (MRI) study (Del Chicca, 2016) were used for this study. Samples from 10 (5 female and 5 male) intact dogs were included in the analysis. One dog was excluded due to a technical problem with the ROTEM device. The dogs were 4 (3 - 6) years old and were weighing 13.7 (10.1 - 15) kg. The dogs were specifically bred for experimental studies and at their animal facility dogs housed in groups of two to three in pens that were 1.45 m x 4.5 m in size with outside runs of approximately 1.45 m x 5.5 m with a minimal height of 2.45 m. Natural daylight was available in the outside run and in the pens through skylights. The dogs were exposed to artificial lighting for 10 h daily with 14 h darkness. Animals were fasted overnight with free access to water up to time of premedication. On the study days the dogs were transported to the study site in a travel cage. The dogs were healthy based on physical exam performed on the day of anesthesia.

Anesthetic management

Dogs were sedated with intramuscular methadone (0.2 mg/kg; Methadon Streuli, Streuli Pharma AG, Switzerland) and were left undisturbed for 20 min before aseptic intravenous catheter (VasoVet 22 gage; Eickemeyer, Switzerland) placement in a cephalic vein. Thirty minutes after sedation the dogs were preoxygenated for 3 min and anesthesia was co-induced with midazolam 0.1 mg/kg (Dormicum, Kantonsapotheke Zurich, Switzerland) and propofol (Propofol 1% MCT Fresenius; Fresenius Kabi AG, Switzerland) intravenously to effect. No heparinized flushing solution was used during the study. The dogs were endotracheally intubated with a cuffed endotracheal tube, connected via a circle breathing system to the anesthetic machine (Aestivia S5, 7900; SmartVent, Switzerland) and placed in dorsal recumbency. Volume-controlled mechanical ventilation with tidal volume of 10 ml/kg was initiated and respiratory frequency adjusted to maintain an end-tidal carbon dioxide between 36 and 46 mmHg (4.8 - 6.1 kPa). Anesthesia was maintained with sevoflurane (Sevorane, AbbVie AG, Switzerland) given to effect in an oxygen and air gas flow of 50 ml/kg/min aiming at an inspired fraction of oxygen of 0.5. Crystalloid fluids (Ringer Lactat Fresenius, Covetrus, Switzerland) were given at 5 ml/kg/h. Cardiovascular and respiratory variables were measured continuously with a multiparameter monitor (Datex-Ohmeda N-MRI2-01; GE Healthcare, Finland) and recorded every 5 min.

Anesthesia maintenance followed a standardized algorithm and was adjusted every 5 min: Non-invasive mean arterial blood pressure (MAP, cuff placement around tail base) was kept between 70 and 80 mmHg in all dogs by adjusting inspired fraction of sevoflurane. In case of MAP < 60 mmHg, the vaporizer dial was decreased by 0.5%. If the MAP stayed persistently < 60 mmHg, an intravenous fluid bolus (2 ml/kg) was administered. If unsuccessful, a dobutamine continuous rate infusion (5

µg/kg/min; Dobutrex, Kantonsapotheke Zurich, Switzerland) was started. In case of MAP > 80 mmHg, the vaporizer dial was increased by 0.5%. The dogs were all recovered successfully after completion of the studies.

Blood collection

Before each blood collection, rectal temperature was measured and recorded. For each dog, 10 ml of blood was collected aseptically from the jugular vein by direct venipuncture with a 20 G needle attached to a 10 ml syringe using minimal vessel occlusion. Blood samples were obtained immediately before induction of anesthesia and before start of fluid administration (T0) and 3.5 h after T0 (T3.5). After sampling, the needle was detached from the syringe and the whole blood was transferred to the corresponding tubes as follows: the first 1.3 ml of the sample were transferred in a serum tube (Sarstedt, Sevelen, Switzerland) for triglyceride measurements followed by 2.9 ml and 1.4 ml blood in sample tubes containing 3.2% Tri-Sodium-Citrate, 1:10 (Sarstedt, Sevelen, Switzerland) for ROTEM and standard plasmatic coagulation parameters, 1 ml in a blood gas syringe containing lyophilized lithium heparin (BD A-Line; Becton, Dickinson and Co., UK) for pH, lactate and ionized calcium measurement, 1.3 ml blood in a potassium-EDTA tube containing 1.6 mg/ml K3 EDTA (Sarstedt, Sevelen, Switzerland) for hematocrit and platelet count. All samples were obtained by the same person, and the specimens were free from macroscopic clots and hemolysis. During the 3.5 h of anesthesia an imaging study (Del Chicca, 2016) was performed.

The contrast agent was only administered after the completion of the current study.

Blood analysis

The following assays were performed at T0 and T3.5: ROTEM activated by proprietary tissue factor (ExTEM), ROTEM activated by ellagic acid and phospholipid (InTEM), EXTEM with added cytochalasin D (FibTEM), and EXTEM with added apoprotein (ApTEM) with a ROTEM-delta thromboelastometer (TEM-International GmbH, Germany). Analysis was performed at 37 °C within 15 min of blood collection by the same operator and according to the manufacturer's instructions and international guidelines (Flatland et al., 2014; Smith et al., 2010). Manufacturer-supplied, lyophilized reagents for the assays were used and the samples were evaluated for 60 min. The following variables were assessed for each profile: clotting time (s), clot formation time (s), α angle (°), maximum clot firmness (MCF, [mm]) and maximum lysis (ML, %). Prothrombin time (PT), activated partial thromboplastin time (aPTT) and fibrinogen concentration using the Claus method (fibrinogen_{Claus}) were determined from plasma after centrifugation (1800 G) using a semi-automated coagulometer (Stat 4, Roche Diagnostics, Switzerland). Platelet number and hemoglobin was measured with fluorescence flow cytometry (Sysmex-XT 2000iV, Sysmex, Japan) and the hematocrit was calculated from hemoglobin. Triglycerides were analyzed by absorption photometry (Cobas C, Roche Diagnostics, Switzerland). The pH, lactate and ionized calcium were measured with the RAPIDpoint 500® (Siemens, Germany). All analyzers underwent routine quality control according to the manufacturers' recommendations. Results were compared to inhouse reference intervals (Bachmann et al., 2018; Jud Schefer et al., 2020).

Statistics

Statistical analysis was performed with a commercially available software (Prism 7.0, Graphpad Software Inc., USA). Results were tested for normality with the Shapiro-Wilk test. Parameters were compared between both time points with paired *t*-test or Wilcoxon matched pairs signed-rank test as appropriate and is presented as median (range). A *p* value < 0.05 was considered significant.

Results

Total infused fluid volume between T0 and T3.5 was 17.5 ml/kg in all dogs and neither additional fluid boli nor dobutamine were administered.

Viscoelastic testing was started 10 (9–12) minutes (T0) and 11 (9–13) minutes (T3.5) after sampling ($p = 0.13$). The ROTEM parameters are summarized in Table 1 and the remaining parameters in Table 2.

Multiple variables changed significantly between T0 and T3.5. The largest changes observed were in body temperature, hematocrit and platelet count. All other significant changes were small (Table 1 and 2) and the values remained within their respective reference intervals.

Table 1

Results of rotational thromboelastometry analysis (ROTEM) activated by proprietary tissue factor (ExTEM) or ellagic acid and phospholipid (InTEM, respectively), ExTEM with cytochalasin D (FibTEM) or aprotinin (ApTEM, respectively) were performed for blood samples from 10 healthy Beagle dogs before induction and after 3.5 h of sevoflurane anesthesia.

	Before induction		After 3.5 h of sevoflurane anesthesia		p-value	Reference interval
	Median	Range	Median	Range		
ExTEM						
Clotting time (s)	35	26 – 57	30*	24 – 46	< 0.01	23 - 87
Clot formation time (s)	117	53 – 153	122	73 – 138	0.79	85 - 357
Alpha angle (°)	68	62 – 82	67	64 – 81	0.84	42 - 77
Maximum clot firmness (mm)	58	51 – 66	57	51 – 64	0.3	32 - 65
Maximum lysis (%)	2	0 – 10	1	0 – 14	>0.99	0 - 12
FibTEM						
Clotting time (s)	47	29 – 278	34*	27 – 120	< 0.01	21 - 112
Maximum clot firmness (mm)	4	3 – 10	5*	3 – 12	< 0.05	2 - 9
ApTEM						
Clotting time (s)	36	31 – 59	32*	27 – 47	< 0.05	21 - 75
Clot formation time (s)	117	67 – 164	117	68 – 216	0.93	99 - 485
Alpha angle (°)	68	59 – 80	67	54 – 82	0.48	42 - 79
Maximum clot firmness (mm)	59	52 – 65	58	48 – 65	0.35	32 - 63
Maximum lysis (%)	1	0 – 3	1	0 – 5	> 0.99	0 - 10
InTEM						
Clotting time (s)	154	123 - 230	165	128 - 205	0.32	133 - 210
Clot formation time (s)	65	45 - 130	69	47 - 93	0.85	59 - 201
Alpha angle (°)	78	66 – 81	76	73 – 81	> 0.99	58 - 78
Maximum clot firmness (mm)	64	57 - 69	63	56 - 69	0.33	52 - 71
Maximum lysis (%)	0	0 - 1	0	0 - 1	1	0 - 3

*Statistically different from T0 ($p < 0.05$).

Discussion

Standardized general anesthesia with concurrent fluid therapy and hypothermia resulted in significant but likely clinically irrelevant changes. The results have to be discussed in the face of a concurrent hypothermia and a decrease in platelet count and in hematocrit.

Extrinsically activated clotting times (ExTEM, ApTEM, FibTEM) were significantly shortened after 3.5 h of anesthesia, while they remained in the reference interval and changes were small. In contrast, PT was not significantly changed, despite good correlation between extrinsic clotting times and PT is reported (Enk et al., 2018; Jud Schefer et al., 2020). The reason for clotting time shortening in extrinsically activated ROTEM assays is unknown and in contrast to the current evidence. *In vitro* hemodilution lead to no change or to a prolongation of viscoelastic clotting times dogs and pigs (Adamik, Butty & Howard, 2015; Mauch et al., 2013; Wurlod et al., 2015) and a recent *in vivo* study administering a comparable amount of crystalloid solutions to dogs undergoing anesthesia for knee surgery did not detect a significant prolongation of clotting times (Reuteler et al., 2016). However, the trauma and inflammation induced by orthopedic surgery may have influenced those results. Our study dogs did not undergo surgery or trauma other than MRI scanning and blood sampling and while the effect of MRI on ROTEM parameters is unknown, we do not consider atraumatic blood sampling to activate coagulation. In contrast, the aPTT was prolonged, while the clotting times of the intrinsically activated ROTEM were unchanged. Again, changes were minimal (Table 2).

The other significant change over time found in ROTEM parameters was a minor but significant increase in FibTEM MCF and a decrease in FibTEM clotting time. An overall hypercoagulable status in face of low hematocrit has been demonstrated in dogs in thromboelastometry assays (McMichael et al., 2014; Smith et al., 2012) and in our study the hematocrit decreased from 49 to 37% (Table 2). However, the maximum clot firmness was not significantly changed by anesthesia and fluid therapy in the three other ROTEM tests (Table 1) that assess clot strength including all blood components (*i.e.* platelets). This different behavior may be explained by the concurrent decrease in platelet count (Table 2). Additionally, volatile agents seem to impair specific platelet receptors *in vitro* (Yuki et al., 2013), but the influence on primary hemostasis has not been investigated in the present study.

In the FibTEM test the platelet function is blocked by cytochalasin D. Therefore, the fibrinogen concentration and function are the primary determinants of the FibTEM MCF and the impact of platelet count on FibTEM MCF is neglectable. This may increase the impact of hematocrit on the results of FibTEM. Of note, fibrinogen concentration measured by Clauss in the current study was significantly lower after 3.5 h of anesthesia (Table 2), which is in contrast to the increase in maximum clot firmness in the FibTEM test (Table 1). Normally a good correlation between fibrinogen measured by Clauss and MCF_{FibTEM} is found (Enk et al., 2018; Jud Schefer et al., 2020). In a study in pigs that were exposed to exsanguination with or without tissue injury, fibrinogen levels were reduced in both groups after exsanguination, but the pigs exposed to injury showed parallel changes to our study (decreased FibTEM clotting time, increased FibTEM maximum clot firmness), compared to the pigs not formerly exposed to injury. It was proposed that extensive tissue trauma might induce an increased fibrin activity attenuating the hypo-coagulable tendency of bleeding pigs (Hagemo et al., 2013). Again, in the current study neither extensive tissue trauma nor blood loss were induced in the examined Beagles.

A recent work determining the correlation between thromboelastography (TEG) variables, packed cell volume and fibrinogen concentration in dogs supports the theory that the observed changes noted with anemia are due to the proportionate increase in available fibrinogen in the fixed microenvironment of the cuvette in the ROTEM (Lynch et al., 2020). Furthermore, another possible explanation for disagreement between FibTEM MCF and fibrinogen levels could be the lack of interference of red blood cell in the polymer net of fibrin in the plasmatic

Table 2

Temperature and results of plasmatic coagulation assays, platelet count, hematocrit, ionized calcium, pH, lactate and triglycerides from 10 healthy Beagle dogs before induction and after 3.5 h of sevoflurane anesthesia.

	Before induction		After 3.5 h of sevoflurane anesthesia		p-value	Reference interval
	Median	Range	Median	Range		
Temperature (°C)	38.3	37.7–38.7	34.9*	34.2–35.8	< 0.0001	
Prothrombin Time (s)	6.9	6.5–7.8	6.9	6.4–7.9	0.91	6.3 - 8.5
Activated Partial Thromboplastin Time (s)	11.5	10.7–13.4	12.6*	10.7–14.3	< 0.05	9.6 - 16.1
Fibrinogen (g L ⁻¹)	2.2	1.4–3.3	1.9*	1.1–3.1	< 0.01	1.0 - 2.5
Platelet Count (10 ³ µL ⁻¹)	314	235–513	258*	207–333	< 0.01	130 - 394
Hematocrit (%)	49	44–59	37*	30–41	< 0.0001	42 - 55
Ionized Calcium (mmol L ⁻¹)	1.32	1.2–1.41	1.34	1.41–1.39	0.41	1.23 - 1.40
pH	7.39	7.36–7.42	7.36*	7.33–7.4	< 0.01	7.32 - 7.45
Lactate (mmol L ⁻¹)	0.95	0.31–1.45	1.37*	0.5–1.83	< 0.01	0.43 - 2.1
Triglycerides (mmol L ⁻¹)	0.4	0.3–1.3	0.3*	0.2–0.5	< 0.01	0.4 - 1.5

*Statistically different from T0 (p < 0.05).

tests (Gersh, Nagaswami & Weisel, 2017).

The coagulation parameter changes have to be interpreted in the face of the decrease in hematocrit and platelet count as well as hypothermia identified at T3.5. The decrease in hematocrit and platelet count can be explained by two concurrent mechanisms. On one hand it has been shown in dogs and monkeys under halothane anesthesia that erythrocytes are sequestered in the spleen, as well as in the splanchnic vasculature, which led to an anesthetic-induced decrease in hematocrit up to 20% (Steffey et al., 1976). This decrease was probably caused by vasodilation and is also expected in newer inhalants such as isoflurane and sevoflurane. Administration of 17.5 ml/kg crystalloid fluids over 3.5 h to an estimated blood volume of 85 ml/kg in these healthy dogs may have diluted the concentration of erythrocytes and platelets to a certain stage (West et al., 2013). The platelet count in dogs has been shown to decrease around 15% after administration of a crystalloid bolus of 10 ml/kg followed by a constant rate infusion of 10 ml/kg for one hour (Chohan et al., 2011). Unfortunately, total solids were not measured in our study to support the hemodiluted effect caused by fluid administration.

The temperature dropped significantly and clinically relevant in the present study from normal values to 34.2 – 35.8 °Celsius. Due to the study setting conducted in a 3 Tesla MRI scanner it was not possible at the time of the study to provide active warming to the dogs. Hypothermia can impair coagulatory function due to several factors such as slowing down of enzymatic processes, platelet adhesion defects, disruption of platelet function, delay of thrombin formation and decreased fibrinogen synthesis (Martini & Holcomb, 2007; Wolberg et al., 2004). An association between hypothermia and coagulation variables in healthy dogs has been shown *in vitro* assessed by TEG (Taggart et al., 2012). Clots formed more slowly at decreased temperatures (27° and 30 °C) but the final strength of the clot appeared unaffected. In temperatures comparable to our results (33 °C and 36 °C), no differences to the control group were noted. There is inconsistency among published reports with respect to the effect of hypothermia on coagulation assessed by TEG or ROTEM in human blood in the literature. Some studies noted hypocoagulable changes in all variables when the temperature of the blood was decreased (Rundgren & Engström, 2008), whereas others did report changes consistent with a decrease in the speed of clot formation and an increase in the clot strength *in vitro* (Ramaker et al., 2009). If the increase in FibTEM MCF in our study could be caused by hypothermia cannot be answered with the results of these studies. *In vitro* results cannot be extrapolated directly to the *in vivo* hypothermic patient as present in the current study. *In vitro* the complex influences of the hypothermic tissue, endothelium and platelets itself are missing (Taggart et al., 2012). Hypothermia of 32 °C decreased fibrinogen synthesis by about 50% with no changes in fibrinogen breakdown measured by stable isotope technique in an *in vivo* swine model (Martini, 2007). Rats subjected to 33 – 34 °Celsius had no alteration in coagulatory function compared to the control group, measured with

ROTEM at the temperature of 37 °C or 33 °C respectively (Park, Lee & Kim, 2013). This is similar to our findings, where ROTEM analysis and standard plasmatic coagulation tests were carried out at 37 °C and not corrected for the dogs' actual rectal temperatures. The difference between the *in vivo* and the *in vitro* analysis temperature may lead to misinterpretation of obtained results. The warming of samples before analysis could eventually have masked hypothermia-induced coagulopathies in the present study. Nevertheless, the authors of a study investigating adjustment of analysis temperature to the *in vivo* value during hypothermia in humans using TEG, concluded that the importance of temperature adjustment in clinical routine is low because of the precision limits of TEG measurement itself (Cundrle et al., 2013). Clot strength and fibrinolysis variables had low ranges of bias both in normothermia and hypothermia. Clot formation variables were influenced by temperature adjustment during hypothermia with wide limits of agreement, which made interpretation of results difficult.

In vitro evaluation of different resting temperatures before analysis of canine whole blood samples revealed that samples rested at room temperature required a longer time to warm up to 37 °C when placed in the reaction cup and therefore the rate of enzymatic reactions was variable. This resulted in a longer lag until thrombin generation, but effects of resting temperature techniques on TEG appear to have a minimal clinical impact (Ralph et al., 2012). The same might apply to hypothermic blood samples. In our study the samples were analyzed within a similar time frame of 9 to 13 min after collection for both the normothermic (T0) and the hypothermic (T3.5) samples. Theoretically the samples from T3.5 starting at a lower temperature had less time to rewarm before analysis. As only the temperature of the device and not the temperature of the blood can be measured during analysis, this cannot be answered with the current study. However, no drop of temperature of the device was displayed during analysis.

The pH stayed within reference range throughout the time course of anesthesia although it decreased significantly between T0 and T3.5 (Table 2). The change of pH can be explained with minor increase in lactate values between T0 and T3.5. A negative influence on coagulation formerly described in a canine trauma model after *in vitro* acidification (Burton et al., 2018) can probably be excluded as the observed changes were minimal.

In the current study triglyceride concentrations were measured to be able to exclude an influence of propofol on the results. Propofol is a lipid-based solution and was used as induction agent. When investigating the *in vitro* effects of supra-clinical doses of lipid emulsion (10 or 30 mg mL⁻¹ concentration) on TEG in canine blood samples, the maximum amplitude (corresponding to maximum clot firmness in ROTEM) was significantly reduced (Tonkin, Parnell & Hogan, 2013). In the present study, triglyceride concentrations dropped slightly (Table 2) and stayed within normal limits at all timepoints, therefore an influence can be excluded.

The current study contains several limitations.

Due to the paired samples and very standardized conditions even very small and probably not clinically relevant differences in variables assessing coagulation reached significance.

With the chosen study design and the lack of total solids measurement, it is not completely possible to differ between the influence of fluid administration and the influence of anesthesia *per se*. However, fluid administration at the chosen rate is common during anesthesia and therefore our results have clinical relevance.

In conclusion, general anesthesia with concurrent haemodilution and hypothermia induced significant but clinically irrelevant changes in coagulation variables measured at 37 °Celsius in healthy Beagle dogs. Blood samples from anaesthetized animals can be used to assess the general coagulation status of dogs.

Ethical statement

This study was approved by the Committee for Animal Experimentation of the Canton Zurich, Switzerland (Nr. 114/2013).

CRedit authorship contribution statement

Andrea Schwarz: Data curation, Formal analysis, Writing – original draft. **Louise F Martin:** Data curation, Writing – review & editing. **Francesca Del Chicca:** Conceptualization, Writing – review & editing. **Nadja E Sigris:** Data curation, Writing – review & editing. **Annette PN Kutter:** Data curation, Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

- Adamik, K.-N., Butty, E., & Howard, J. (2015). *In vitro* effects of 3% hypertonic saline and 20% mannitol on canine whole blood coagulation and platelet function. *BMC Veterinary Research*, pp.1–9.
- Agarwal, S., & Abdelmotieleb, M. (2020). Viscoelastic testing in cardiac surgery. *Transfusion*, 60(6), 52–60.
- Bachmann, K., et al. (2018). Determination of reference intervals and comparison of venous blood gas parameters using a standard and non-standard collection method in 51 dogs. *Schweizer Archiv für Tierheilkunde*, 160(3), 163–170.
- Boyd, C. J., et al. (2018). Hypocoagulability and platelet dysfunction are exacerbated by synthetic colloids in a canine hemorrhagic shock model. *Frontiers in Veterinary Science*, 5, 588–611.
- Burton, A. G., et al. (2018). *In vitro* assessment of the effect of acidemia on coagulation in dogs. *Journal of Veterinary Emergency and Critical Care*, 28(2), 168–172.
- Chohan, A. S., et al. (2011). Effects of 6% hetastarch (600/0.75) or lactated Ringer's solution on hemostatic variables and clinical bleeding in healthy dogs anesthetized for orthopedic surgery. *Veterinary Anaesthesia and Analgesia*, 38(2), 94–105.
- Cundrie Jr., I. et al., (2013). Temperature corrected thromboelastography in hypothermia. Is it necessary? *European Journal of Anaesthesiology*, 30(2), pp.85–89.
- Del Chicca, F., et al. (2016). Perfusion- and diffusion-weighted magnetic resonance imaging of the liver of healthy dogs. *American Journal of Veterinary Research*, 77, 463–470.
- Enk, N. M., et al. (2018). Correlation of plasma coagulation tests and fibrinogen clauswith rotational thromboelastometry parameters and prediction of bleeding in dogs. *Journal of Veterinary Internal Medicine*, 96, 711–719.
- Flatland, B., et al. (2014). Systematic evaluation of evidence on veterinary viscoelastic testing Part 2: Sample acquisition and handling. *Journal of Veterinary Emergency and Critical Care*, 24(1), 30–36.
- Fry, W., et al. (2017). Thromboelastography in Dogs with Chronic Hepatopathies. *Journal of Veterinary Internal Medicine*, 31(2), 419–426.
- Gersh, K., Nagaswami, C., & Weisel, J. (2017). Fibrin network structure and clot mechanical properties are altered by incorporation of erythrocytes. *Thrombosis and Haemostasis*, 102(12), 1169–1175.
- Hagemo, J. S., et al. (2013). Changes in fibrinogen availability and utilization in an animal model of traumatic coagulopathy. *Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine*, 21(1), 1.
- Herrero, Y., et al. (2020). Serial evaluation of haemostasis following acute trauma using rotational thromboelastometry in dogs. *Veterinary and Comparative Orthopaedics and Traumatology*, 29(05), 6–8.
- Jud Schefer, R., et al. (2020). Determination of reference intervals for single vial rotational thromboelastometry (ROTEM) parameters and correlation with plasmatic coagulation times in 49 clinically healthy dogs. *Research in Veterinary Science*, 129, 129–136.
- Kelley, D., et al. (2015). Thromboelastographic evaluation of dogs with acute liver disease. *Journal of Veterinary Internal Medicine*, 29(4), 1053–1062.
- Kol, A., & Borjesson, D. L. (2010). Application of thrombelastography/thromboelastometry to veterinary medicine. *Veterinary Clinical Pathology*, 39(4), 405–416.
- Koo, B.-W., et al. (2014). The influence of propofol and sevoflurane on hemostasis: A rotational thromboelastographic study. *Anesthesia Pain Medicine*, 9, 292–297.
- Lynch, A. M., et al. (2020). The influence of packed cell volume versus plasma proteins on thromboelastographic variables in canine blood. *Journal of Veterinary Emergency and Critical Care*, 30(4), 418–425.
- Martini, W. Z., & Holcomb, J. B. (2007). Acidosis and Coagulopathy. *Annals of Surgery*, 246(5), 831–835.
- Mauch, J., et al. (2013). Effect of rapid fluid resuscitation using crystalloids or colloids on hemostasis in piglets. *B. Anderson, ed. Pediatric Anesthesia*, 23(3), 258–264.
- McMichael, M. A., et al. (2014). *In vitro* hypercoagulability on whole blood thromboelastometry associated with *in vivo* reduction of circulating red cell mass in dogs. *Veterinary Clinical Pathology*, 43(2), 154–163.
- Park, K. H., Lee, K. H., & Kim, H. (2013). Effect of hypothermia on coagulatory function and survival in Sprague-Dawley rats exposed to uncontrolled hemorrhagic shock. *Injury*, 44(1), 91–96.
- Ralph, A. G., et al. (2012). Effects of rest temperature, contact activation, and sample technique on canine thrombelastography. *Journal of Veterinary Emergency and Critical Care*, 22(3), 320–326.
- Ramaker, A. J., et al. (2009). Effects of acidosis, alkalosis, hyperthermia and hypothermia on haemostasis: Results of point of care testing with the thromboelastography analyser. *Blood Coagulation & Fibrinolysis*, 20(6), 436–439.
- Reuteler, A., et al. (2016). Comparison of the effects of a balanced crystalloid-based and a saline-based tetrastarch solution on canine whole blood coagulation and platelet function. *Journal of Veterinary Emergency and Critical Care*, 27(1), 23–34.
- Rundgren, M., & Engström, M. (2008). A Thromboelastometric Evaluation of the Effects of Hypothermia on the Coagulation System. *Anesthesia & Analgesia*, 107(5), 1465–1468.
- Sayce, A. C., Neal, M. D., & Leeper, C. M. (2020). Viscoelastic monitoring in trauma resuscitation. *Transfusion*, (60), 33–51.
- Sigris, N. E., et al. (2017). Hyperfibrinolysis and Hypofibrinogenemia Diagnosed With Rotational Thromboelastometry in Dogs Naturally Infected With *Angiostrongylus vasorum*. *Journal of Veterinary Internal Medicine*, 31(4), 1091–1099.
- Smith, S.A. et al., (2010). Clot formation in canine whole blood as measured by rotational thromboelastometry is influenced by sample handling and coagulation activator. *Blood Coagulation & Fibrinolysis*, pp. 1–11.
- Smith, S. A., et al. (2012). Correlation of hematocrit, platelet concentration, and plasma coagulation factors with results of thromboelastometry in canine whole blood samples. *American Journal of Veterinary Research*, 73(6), 789–798.
- Steffey, E. P., et al. (1976). Effect of halothane and halothane-nitrous oxide on hematocrit and plasma protein concentration in dog and monkey. *American Journal of Veterinary Research*, 37(8), 959–962.
- Taggart, R., et al. (2012). *In vitro* evaluation of the effect of hypothermia on coagulation in dogs via thromboelastography. *Journal of Veterinary Emergency and Critical Care*, 22(2), 219–224.
- Tonkin, L. R., Parnell, N. K., & Hogan, D. F. (2013). *In vitro* effects of lipid emulsion on platelet function and thromboelastography in canine blood samples. *American Journal of Veterinary Research*, 74(4), 567–571.
- West, E., et al. (2013). Acid-base and electrolyte balance following administration of three crystalloid solutions in dogs undergoing elective orthopaedic surgery. *Veterinary Anaesthesia and Analgesia*, 40(5), 482–493.
- Wolberg, A. S., et al. (2004). A systematic evaluation of the effect of temperature on coagulation enzyme activity and platelet function. *The Journal of Trauma: Injury, Infection, and Critical Care*, 56(6), 1221–1228.
- Wurlod, V. A., et al. (2015). Comparison of the *in vitro* effects of saline, hypertonic hydroxyethyl starch, hypertonic saline, and two forms of hydroxyethyl starch on whole blood coagulation and platelet function in dogs. *Journal of Veterinary Emergency and Critical Care*, 25(4), 474–487.
- Yuki, K., et al. (2013). Volatile anesthetics, not intravenous anesthetic propofol bind to and attenuate the activation of platelet receptor integrin $\alpha\text{IIb}\beta\text{3}$. *Cheng, ed. PloS one*, 8(4), e60415–e604110.