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Physiological effects of rapid intravenous infusion of fluids for the treatment of hypovolaemic hypotension in anaesthetised cats

By

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in the Faculty of Health Sciences, University of Witwatersrand

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

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DECLARATION

I, Gareth E. Zeiler, hereby declare that the research presented in this thesis, was conceived and executed by myself, under guidance from my supervisors.

Neither the substance, nor any part of the thesis has been submitted in the past, or is to be submitted for a degree at the University of Witwatersrand or any other University.

This thesis is presented for fulfilment of the requirements for degree of Doctor of Philosophy (PhD).

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THESIS ABSTRACT

Physiological effects of rapid intravenous infusion of fluids for the treatment of hypovolaemic hypotension in anaesthetised cats

My research focused on providing information to further our understanding on the physiology of severe haemorrhage and the administration of fluids to resuscitate the intravascular compartment in anaesthetised domestic cats. To date, there are very few reports detailing evidence-based treatments on how to manage cats in these clinical situations. The current treatment recommendations are based on decades of anecdotal textbook-based evidence, or are recommendations extrapolated from dogs but adjusted to fit cat-related differences. Although current treatments are effective in many cats, the risk of complications is also very high. Fluid overload occurs commonly during fluid resuscitation. Therefore cats are frequently claimed to be a 'fluid-sensitive' species and caution is strongly advised when administering intravenous fluids during hypovolaemic resuscitation. This fluid-sensitive sentiment can easily lead to under-treatment or, if complications arise during management, attribution of blame to the fluids used as the most likely cause of treatment failure. These situations can delay or prevent a cat from receiving the correct treatment as soon as possible to prevent lingering shock, or can result in aggressive overtreatment and fluid overload and its sequalae. A comprehensive understanding of the cat's physiology during severe haemorrhage and fluid resuscitation will demystify fluid treatment protocols and allow for clinically meaningful recommendations to be made.

This thesis comprises of a series of investigations which focus on physiological responses to: 1) acute severe haemorrhage and 2) fluid administration to treat haemorrhage-induced hypovolaemic-hypotension. Six domestic cats underwent three treatments, two month apart, in a randomised crossover pattern. The cats were anaesthetised using: 1) buprenorphine as a pre-anaesthetic medication, 2) alfaxalone for induction into general anaesthesia, and 3) isoflurane in oxygen for maintenance of general anaesthesia. The three treatments were: 1) control, with sham haemorrhage and resuscitation; 2) controlled haemorrhage followed by lactated Ringer's solution for resuscitation (LRS); and 3) controlled haemorrhage followed by tetrastarch 6% 130/0.4 for resuscitation. Cats that underwent a controlled haemorrhage phase, followed by a resuscitation phase were administered LRS or tetrastarch 6% 130/0.4 at 60- and 20-ml kg^{-1} per hour, respectively, for 120 minutes. The endpoint of haemorrhage was a 1) maximum of 30 ml kg^{-1} of blood withdrawn, or 2) invasively measured mean

arterial blood pressure < 48 mmHg for 3 minutes. This controlled haemorrhage model in cats while under general anaesthesia emulates a clinically relevant scenario, whereby cats undergoing surgical procedures could haemorrhage and the surgeon could gain control of the haemorrhage.

The first study in this thesis describes changes in physiological, haematological and biochemical variables following a mild and severe haemorrhage event. Variables that changed significantly were further analysed to determine if they could be used in a scoring system aimed to quantify acute haemorrhage during general anaesthesia. We found that easily determinable variables could be placed into four ratios that, when analysed together, can reliably quantify acute blood loss in anaesthetised cats. We called this scoring system the Cat Acute Bleeding Scoring System, or CABSS as an abbreviated term. The second study in this thesis describes changes in physiological, haematological and biochemical variable values during the fluid resuscitation phase where LRS or tetrastarch 6% 130/0.4 were administered. Bearing in mind that cats are regarded as fluid-sensitive, we specifically aimed to derive volume-endpoints to guide how much fluid should be administered to cats that have just undergone an acute severe haemorrhage. Volume-endpoints are unlike resuscitation endpoints which are commonly derived cardiovascular or oxygenation biomarkers used to guide fluid therapy in goal-directed fluid administration protocols. Resuscitation endpoints seek to restore normal physiological function, however, the lack of clear guidelines on how much fluid to administer before deciding a cat is a fluid non-responder predisposes them to fluid overload. During resuscitation, fluids are often required to fill the intravascular compartment, but there are no clear guide for veterinarians to stop administering fluids and rather begin other treatment modalities, such as administration of a catecholamine to restore and meet traditional resuscitation endpoint targets. My thesis is the first to describe such endpoints in cats that have undergone severe haemorrhage. We found that changes in the sodium, chloride and magnesium electrolyte concentrations were helpful in determining volume-endpoints. Specifically, the chloride to sodium ratio appears to be the volume-endpoint of choice and is easy to measure and cost-effective, unlike many of the traditional resuscitation endpoints. The clinical goal is to administer resuscitation fluids until the volume-endpoint is reached; and if the cat still does not meet the desired resuscitation endpoint, then catecholamines or other cardio-active drugs should be considered before administering more fluids, to prevent fluid overload. The third study describes the acid-base status of the cats during haemorrhage and resuscitation. We made use of three methods to analyse the acid-base status, namely: 1) Henderson-Hasselbalch, 2) Stewart, and 3) semi-quantitative

methods. We found that severe haemorrhage and large volume resuscitation did not alter the blood pH, and acidosis was only detected during anaesthesia. Despite the pH being similar among treatments, there were method-associated differences in variables used to interpret pH. However, the clinical relevance of the acidosis is not yet fully understood. The fourth study describes the haemostatic effects of severe haemorrhage followed by fluid resuscitation. We found that haemostatic derangements occurred during the resuscitation phase of the LRS and tetrastarch 6% 130/0.4 treatments, and we attributed these to haemodilution.

The findings of these studies have clinical relevance and allow us to quantify acute intraoperative haemorrhage and guide fluid resuscitation, using two commonly available intravenous fluids for treatment of haemorrhage-induced hypovolaemia. In order to improve our management of cats during the peri-anaesthetic period, we require evidence-based treatment guides and protocols which are aimed at improving the overall outcome of treating hypovolaemia in cats. It is essential that the CABSS and volume-endpoints be scrutinised and evaluated on a larger and more diverse population of cats requiring treatment for other pathophysiological presentations besides during the peri-anaesthetic period. The evidence provided in this thesis improves our overall understanding of the physiology of severe haemorrhage followed by fluid resuscitation, especially during the peri-anaesthetic period.

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I would like to thank Marleze Rheeder, Ilse Janse van Rensburg and Antoinette van Wyk and their team at the University of Pretoria Biological Research Centre (UPBRC) for rearing and caring for the cats during the study. I would also like to thank Drs Roxanne Buck and Friederike Pohlin that visited the cats on a regular basis and assisted during the data collection phase of the project and helped in the rehoming adoption process. Also, Karien Muller for her organisation and management of the samples reaching the Onderstepoort Veterinary Academic Hospital Clinical Pathology Laboratory.

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LIST OF ABBREVIATIONS

Abbreviation(s)	Meaning
≈	Almost equal to or approximately equal to
+LR and -LR	Positive and negative likelihood ratios
+PV and -PV	Positive and negative predictive values
α-angle	Alpha-angle
°C	Degree(s) Celsius
-15, 0, 30, 60, 90, 120	Time periods before haemorrhage (-15), after haemorrhage and start of resuscitation (0), and at 30, 60, 90 and 120 minutes during resuscitation
ADP	Adenosine triphosphate
AG	Anion gap
ANOVA	Analysis of variance
aPTT	Activated partial thromboplastin time
Atot	Total weak acids
AUC	Area under the curve
BE(ecf) or BE(B)	Base excess of extracellular fluid or base excess of blood
Beats min^{-1}	Heart beats per minute
Breaths min^{-1}	Breaths per minute
Ca^{++}	Calcium ion
CABSS	Cat Acute Bleeding Scoring System
Cl^{-}	Chloride ion
CL30 and CL60	Clot lysis at 30 and 60 minutes
CO_2	Carbon dioxide
ECM	Extracellular matrix
e.g.	Example
ET	Endotracheal
ET-tube	Endotracheal tube
$F_E´O_2$	Fractional exhalation of oxygen
Fg	Fibrinogen
FiO_2	Fractional inspiration of oxygen
f_R	Respiratory rate
g dL^{-1} and g L^{-1}	Gram(s) per decilitre and Gram(s) per litre
GPIb-XI-V	glycoprotein Ib
GPVI	Glycoprotein VI receptors
G-value	Global value
H^{+}	Hydrogen ion
H1	Histamine receptors type 1
Hb	Haemoglobin concentration

HC	Health check
HCO_3^-(act) or HCO_3^-(std)	Actual or standard bicarbonate ion concentration
$[HCO_3^-]:[H_2CO_3]$	Bicarbonate ion to carbonic acid ratio
H_2CO_3	Carbonic acid
H_2O	Water
HR	Heart rate
Ht	Haematocrit
im	Intramuscular
IQR	Interquartile range
iv	Intravenous
K^+	Potassium ion
Ka	Effective equilibrium dissociation constant
Kg	Kilogram
kPa	Kilo Pascal(s)
K-time	Kinetic time
L L^{-1}	Litre per litre
L min^{-1} or L $minute^{-1}$	Litre(s) per minute
log_{10}	Logarithm
LRS	Lactated Ringer's solution
Ly 30 and Ly 60	Lysis time at 30 and 60 minutes
MA	Maximum amplitude
MAP	Mean arterial blood pressure
Max	Maximum
Mean	Average
mEq	Milliequivalence(s)
mEq L^{-1}	Milliequivalence(s) per litre
mg	Milligram(s) reported as a total dose
mg dL^{-1}	Milligram(s) per decilitre
mg kg^{-1}	Milligram(s) per kilogram
mg kg^{-1} $hour^{-1}$	Milligram(s) per kilogram per hour
mg ml^{-1}	Milligram(s) per millilitre
Min	Minimum
ml	Millilitre(s)
ml kg^{-1}	Millilitre(s) per kilogram
ml kg^{-1} $hour^{-1}$	Millilitre(s) per kilogram per hour
ml $minute^{-1}$	Millilitre(s) per minute
mmHg	Millimetre(s) Mercury
mmol	Millimole(s)
mmol L^{-1}	Millimole(s) per litre
MODS	Multiple organ dysfunction syndrome

n	Number of (count of)
Na^+	Sodium ion
ND or NDay	Next day or day after treatments (approximately 24 hours later)
nmol L^{-1}	Nanomole(s) per litre
NSAIDs	Non-steroidal-anti-inflammatory drugs
OH^-	Hydroxide ion
p	Level of statistical significance
$PaCO_2$	Arterial partial pressure (or tension) of carbon dioxide
$PaCO_2$ - $P_E´CO_2$ or $P(a-_E´)CO_2$	Arterial to end-tidal carbon dioxide tension gradient
PAO_2	Alveolar partial pressure (or tension) of oxygen
PaO_2	Arterial partial pressure (or tension) of oxygen
$P(A-a)o_2$	Alveolar-to-arterial oxygen tension gradient or A-a gradient
Patm or Pbar	Barometric (or atmospheric) air pressure
$P_E´CO_2$	Partial pressure of expired carbon dioxide
pH	Negative logarithm of the Hydrogen ion concentration
PH_2O	Partial pressure (or tension) of water or alveolar vapour pressure
pKa (or pK_1)	Equilibrium dissociation constant (of carbonic acid)
PT	Prothrombin time
R	Correlation coefficient
RCC	Red cell count
ROC	Receiver operating characteristic
RQ	Respiratory quotient
R-time	Reaction time
S	Solubility coefficient of carbon dioxide in plasma
SaO_2	Arterial oxygen-haemoglobin saturation (Calculated)
sc	Subcutaneous
SD	Standard deviation
SID	Strong ion difference
SIDa	Apparent strong ion difference
SIDe	Effective strong ion difference
SIG	Strong ion gap
SIRS	Systemic inflammatory response syndrome
SpO_2	Peripheral oxygen-haemoglobin saturation
TEG	Thromboelastography
Temp	Temperature
Vd/Vt	Dead-space to tidal volume ratio
Ve	Minute volume
Vol	Voluven which is 6% tetrastarch 130/0.4 suspended in normal saline
VTexp	Expiratory tidal volume
vWf	von Willebrand factor

CHAPTER 1

General introduction, literature review and scope of the thesis

1.1 General introduction

The intravascular compartment is made up of the heart and the vascular tree (veins and arteries) that interact with body tissues at the level of the capillary bed (Davis et al. 2013, Lunn et al. 2012). The main function of the cardiovascular system is the transportation of substances to and from the body tissues. The most important substance transported by the cardiovascular system is oxygen, which is required to produce energy through aerobic metabolism by body tissues (DiBartola 2012). Whole blood is made up of a cellular (erythrocytes, leukocytes, thrombocytes) component and a fluid component (plasma); which offers various methods of transporting substances. The circulating whole blood volume in a healthy, hydrated, normal body condition cat is approximately 6 to 7% of its body mass, or estimated to be 62 to 66 mL kg^{-1} body mass (Davis et al. 2013, Lunn et al. 2012). However, some past studies report the measured circulating whole blood volume of cats is a mean (± SD) of 52.6 ± 6.8 mL kg^{-1} (Groom et al. 1965; Mott 1968). The intravascular volume is maintained through various physiological mechanisms. If a depleted intravascular volume is not restored, metabolically active tissue beds may not be adequately perfused and thus hindering aerobic metabolism. If poor perfusion of tissue beds persists, a systemic inflammatory response syndrome (SIRS) that is often the prequel of multiple organ dysfunction syndrome (MODS) and death may ensue (DiBartola 2012, Driessen & Brainard 2006).

Clinically, a depleted intravascular volume can be replaced by different resuscitation fluids. The rate and volume of administration of these fluids will depend on the clinical presentation and type of fluid lost in the patient (Davis et al. 2013, Lunn et al. 2012). Various resuscitation fluids have been used to treat hypotension in veterinary patients, including: isotonic crystalloids (e.g. lactated Ringer's solution), hypertonic solutions (e.g. 7.5% hypertonic saline [sodium chloride]), synthetic colloids (e.g. hydroxyethyl starches), haemoglobin-based fluids with colloidal properties (e.g. bovine haemoglobin glutamer-200) and natural colloids (e.g. fresh whole blood) (Davis et al. 2013, DiBartola 2012, Driessen & Brainard 2006). Despite clear evidence of clinical use of various fluids for cardiovascular volume resuscitation in cats, there is a lack of evidence-based research to safely advocate a fluid resuscitation

intervention in anaesthetised cats that suffer severe acute haemorrhage while undergoing a surgical procedure. The effects of the various fluids on haemostasis, electrolyte balance and arterial oxygen content are lacking. The two most commonly prescribed fluid types, based on clinical experience, are isotonic crystalloids and synthetic colloids belonging to the hydroxyethyl starches; these fluids will be the focus of the thesis.

Isotonic crystalloids have been used as resuscitation fluids in cats (Davis et al. 2013, Kastner 2014, Lunn et al. 2012, Mazzaferro & Powell 2013, Pascoe 2012). The recommended volume of infusion ranges from 50 to 75 mL kg^{-1} administered over 20 to 60 minutes, depending on how rapidly the clinician needs to restore the intravascular compartment (Davis et al. 2013, Kastner 2014, Lunn et al. 2012, Mazzaferro & Powell 2013, Pascoe 2012). The recommended replacement volume in cats seems to be based on extrapolations from concepts of dog fluid resuscitation; yet the circulating volume of blood ranges from 62 to 66 mL kg^{-1} in cats, compared to 75 to 90 mL kg^{-1} in dogs (Wellman et al. 2012). Thus the resuscitation volume is recalculated to the proportionately equivalent circulating blood volume in cats. Anecdotally, the extrapolated concept appears to be valid. However, Brodbelt (2010) reported that cats that receive fluids under anaesthesia are four times more likely to die compared to those that did not. Excessive isotonic crystalloid fluid administration that perpetuates inadvertent fluid overload is a chief concern in cat anaesthesia (Brodbelt 2010). Clinical experience also suggests that fluid overload may be an accidental complication during intensive care management of cats (Mazzaferro & Powell 2013).

Synthetic colloids (mainly hydroxyethyl starches) have been used for their plasma expansion effects in cats. They are often administered as boluses. The recommended boluses in cats are between 3 and 10 mL kg^{-1} (Davis et al. 2013, Lunn et al. 2012, Mazzaferro & Powell 2013, Hughes & Boag 2012, Holbeck & Grande 2000), that can be repeated twice in a 24 hour period. These volumes are low compared to those recommended for dogs (10-20 mL kg^{-1}). These lower volumes are perhaps based on clinical experience and the knowledge that cats have a relatively lower circulating fluid volume. Furthermore, cats are thought to develop volume overload more easily compared to dogs (Davis et al. 2013). However, there is no evidence that these volumes are efficacious or safe in cats.

A haemorrhage-induced hypovolaemia and hypotension model used in isoflurane-anaesthetised dogs has been reported previously (Muir & Wiese 2004). In this model, a volume of blood was withdrawn

(total calculated at the end of the procedure) to cause hypotension (systolic arterial blood pressure < 80 mmHg). The blood withdrawal was done over a 15 minute period. The dogs were left in the hypotensive state for 20 minutes before the resuscitation fluid (lactated Ringer's solution or 6% hetastarch solution) was administered. Both the resuscitation fluids were administered at a fixed rate (90 mL kg^{-1} per hour) until the systolic arterial blood pressure was restored to within 10% of the pre-haemorrhage value. The total amount of resuscitation fluid required was calculated. No effort was made to adjust the end-tidal isoflurane concentration in this study, despite haemorrhage-induced hypovolaemia causing a decrease in the minimum alveolar concentration (Mattson et al. 2006). A similar study model was proposed by us for the series of studies that were conducted in cats and reported in this thesis. Instead of using the systolic blood pressure as a marker for hypotension, we used the mean arterial blood pressure measured directly using invasive blood pressure measurements. Direct mean arterial blood pressure is a better indicator of hypotension and volume status compared to the systolic blood pressure (DiBartola 2012). The intravascular physiology, and perhaps anatomy, of the dog is different to that of a cat. The species-relevant differences include their different circulating volumes of blood: 75 to 90 mL kg^{-1} in dogs compared to an estimated range of 62 to 66 mL kg^{-1} in cats (Wellman et al. 2012). Furthermore, dogs are more tolerant of intravascular volume loading; and a number of proposed, yet unproven, explanations for this tolerance include: 1) increased ability of vasodilation, therefore, allowing the intravascular volume to accumulate in the venous system and splanchnic organs (liver and spleen); 2) improved renal capacity to control total water balance; 3) increased fluid loss via the colon; 4) different endothelial anatomy (glycocalyx structure) that is more resistant to disruption after fluid resuscitation (Hobeck & Grande 2000; Hughes & Boag 2012; Lunn et al. 2012). Species differences in fluid resuscitation in carnivores are evident and simple extrapolation for cats from dog fluid resuscitation strategies is fraught with difficulty and not recommended.

There is a lack of scientific information on acute fluid resuscitation regimes in cats. Many of the recommended infusion and bolus rates of the fluids are extrapolated from dog studies. These extrapolations seem to be inferred values based on the circulating volume of cats. Evidence-based knowledge on fluid resuscitation remains a clinical challenge in cats where fluid overload is arguably a great concern. Literature on blood coagulation perturbations, arterial oxygen content, electrolyte shifts and acid-base alterations during rapid fluid resuscitation in cats is also lacking. This thesis aimed to

address these deficiencies in our knowledge and hopefully answer fundamental questions that remain unanswered when using resuscitative fluid therapies in anaesthetised domestic cats.

1.2 Review of the physiology of body fluid balance in domestic cats

1.2.1 Physiology of the cardiovascular and respiratory systems and blood

This thesis provides details on cats that underwent haemorrhage followed by resuscitation using two different types of fluids. We monitored the cats carefully during the study procedures, especially responses in the cardiovascular and respiratory systems and blood. Therefore, we make mention of fundamental physiology which was important in topic of this thesis.

1.2.1.1 Physiology of the cardiovascular system

The cardiovascular system of cats comprises of two blood vessel circuits (systemic and pulmonary) connected by a four-chambered heart (two atria and two ventricles) (Cobb 1994). This system's main function is transportation of mainly oxygen, nutrients and metabolic by-products to and from the metabolising tissues throughout the body. The heart pumps blood into the two circuits simultaneously through cyclical contraction of filled ventricles (systole) and relaxation during ventricular filling (diastole). The right side of the heart receives blood from the systemic circuit and pumps it into the pulmonary circuit; the right atrium receives blood returning to the heart via the vena cava which then flows into the right ventricle which pumps the blood into the pulmonary artery (Cobb 1994). The left side of the heart receives blood from the pulmonary circuit and pumps it into the systemic circuit; the left atrium receives blood from the pulmonary vein which then flows into the left ventricle which pumps the blood into the aorta (Cobb 1994). The blood vessels of each circuit are similar in anatomical design and start as blood is pumped out of the ventricle of the heart into the large diameter major arteries which branch out into narrower arterioles which then divide to form capillary beds (where gas and nutrient and by-product exchange occurs with the metabolising tissues); on exiting the capillary bed, the blood enters the

venules which drain in larger major veins on their way back to the atria of heart (Cobb 1994). At any given time, approximately 16% of the blood volume is within the arterial side of the systemic circuit, 4% within the heart, pulmonary circuit and capillary beds. The majority of blood (64%) is within the venous side of the systemic circuit. The right side of the heart is a low-pressure system whereas the left side of the heart is a high-pressure system. Blood, which is a fluid, relies on the generation of a pressure gradient to induce flow from a high to a low pressure. Therefore, the arterial sides of the circuits are the starting point of blood flow and must generate enough pressure to ensure the blood makes it throughout the respective circuits. The venous side, after the capillary beds, has a much lower operating pressure, but still relies on a pressure gradient to encourage flow back towards the atria of the heart (the lowest pressure within the circuits). The relationship between flow and pressure can best be described in the simplest terms by reapplying the Ohm's Law electrical equation as a fluid dynamic equation as follows (Brown 2012):

$$\text{Blood flow} = \frac{\text{Pressure gradient}}{\text{Vascular resistance}}$$

However, the pressure dynamics within the arterial and venous side of the circuits are different and not as simple as the Ohm's Law equation predicts. Within the arterial side of the circuit, the arterioles are the major contributors to increasing vascular resistance (vasoconstriction); this is the region within the arterial side where pressure is generated and there are three relevant pressure measurements to consider, the diastolic (DAP), systolic (SAP) and mean (MAP) arterial blood pressures. In cats, the reference interval for DAP is 70-120 mmHg, SAP is 120-170 mmHg, and MAP is 60 to 130 mmHg measured directly (Waddell 2000). The DAP can be considered the resting pressure within the arterial side of the circuit when the heart is relaxed and not pumping blood. The SAP is the most active pressure which reflects the dynamic relationship of the ventricles pushing blood into an elastic type system which is already filled with blood. The MAP is the overall driving pressure within the arterial side of the circuits and its contribution to the pressure gradient can be calculated by calculating the pulse pressure (difference of SAP and DAP) then dividing this number by 3 and then adding that number to DAP (MAP = [(SAP-DAP)/3] + DAP) (Sierra & Savino 2015). As the heart beats and pumps blood into the arteries during systole (heart contraction phase) the blood flows in a pulsatile manner where there is constant flow during diastole (heart resting phase), but increased flow as a pulse wave during systole (Cobb 1994). The major arteries are very elastic and are already distended because of the diastolic pressure;

so when additional blood enters the system during systole, the arteries expand locally. Once the artery expands; due to its elastic nature, it will want to return to its resting diastolic state, which then generates a 'peristaltic wave' along the artery from the heart towards the capillary beds. This dynamic relationship between the heart and the arteries generates pulsatile flow and this relationship is called the Windkessel effect (Cappello et al. 1995). Due to the pulsatile nature of blood, the pressure fluctuates from DAP to SAP, and the following equation can be used to interpret the determinants of pressure (Mazzaferro & Wagner 2001):

$$\text{Arterial blood pressure} = \text{Cardiac output x Vascular resistance}$$

The diastolic pressure is measured when there is no contraction of the heart and therefore no cardiac output. Therefore this pressure is generated by the arterial side being filled to its natural capacity when there is normal vessel tone (not vasodilated or vasoconstricted). The pressure during diastole is generated by the returning of the blood vessels, after the elastic expansion, to their resting state and the tone of the arteriole (the resistance generating part of the arterial side). For this pressure to be generated, the arterial side must be full (blood pushes up again wall of vessel) and there must be a normal vessel tone (vessel pushing back onto blood) and a normal tone in the arteriole to maintain normal flow into the capillary beds (Boyd & Smart 2018). The systolic pressure is related to the cardiac output and how this output stretches the arteries over and above the normal resting tone within the vessel. The determinants of cardiac output can be interpreted using the following equation:

$$\text{Cardiac output} = \text{Stroke volume x Heart rate}$$

The stroke volume of the heart is how much blood is ejected from the ventricle and is thus dependent on three major factors: 1) the preload, 2) the inotropic capacity of the heart, and 3) the afterload (Mazzaferro & Wagner 2001). The preload is the amount of blood that fills and stretches the ventricles immediately before systole begins, therefore it is dependent on how much blood is returning to the heart via the venous system (venous return). The final phase of ventricular filling is because of the atrial kick which is when the atria contract and push the final amount of blood into the ventricle before systole (Cobb 1994). The volume transferred during the atrial kick is also dependent on how much volume of blood returns to the heart. During a resting state, blood is continually moving from the arteriole, through the capillary beds and into the venules; this flow is enough to generate flow back to the heart. The major

veins have one-way valves, which ensure that blood is continually moving towards the heart (Cobb 1994). During exercise, skeletal muscle contractions increase the pressure within the venous system and help to generate a larger pressure gradient and improved venous return. This is called the skeletal muscle pump. Similarly, the thoracic cavity is under fluctuating pressure condition, and during the respiratory cycle, there is a change in venous return, especially if the animal is volume depleted. During spontaneous inspiration (more negative pressure within the thoracic cavity) the venous return is greater compared to during the expiratory phase (less negative pressure within the thoracic cavity). This is called the respiratory pump. Veins are also considered the capacitance vessels, and therefore under certain conditions, like haemorrhage, the circulatory system will recruit blood from this venous reservoir to maintain an adequate venous return and thus preload. The inotropic capacity of the heart is how strongly the myocardium contracts during systole (Cooper 2018). The stronger the contraction, the greater the pressure generated within the ventricle; and the larger the volume of blood that will be ejected into the arterial side of the circuits. There are factors that can alter the inotropic capacity of the heart. The most important regulator of inotropy is the autonomic nervous system. The sympathetic branch of the autonomic nervous system innervates the atria and ventricles and, when activated, causes an increase in inotropy (positive inotropic effect). The parasympathetic branch of the autonomic nervous system mainly innervates the atria and has a minor effect in causing a decrease in inotropy (negative inotropic effect). High levels of circulating catecholamines (adrenaline and noradrenaline) augment the sympathetic nervous system's effect and increase the inotropic capacity of the heart (Cobb 1994). Furthermore, the sympathetic nervous system and the circulating catecholamines cause an increase in heart rate too; and the increase in heart rate also stimulates an increase in the inotropic capacity, regardless of the cause. When an increase in heart rate causes an increase in inotropic capacity, then this effect has been termed the treppe effect (staircase effects) or Bowditch effect or frequency-dependent inotropy (Noble 1988). This effect is thought to occur when the sodium ion influx into the heart muscles is too high and exhausts the sodium-potassium exchange pump (Na-K ATPase); the intracellular sodium is then removed from the cytosol by the sodium-calcium exchange pump which leads to a high intracellular calcium content, which is the stimulation for the increased inotropic capacity (Noble 1988). Additionally, when the sympathetic nervous system is activated, or when catecholamines are released systemically, vasoconstriction occurs within the arterioles, which increases the systemic vascular resistance and afterload (discussed later). The increase in afterload is also a stimulation for

the heart to increase its inotropic capacity and this is called the Anrep effect (von Anrep 1912). Afterload, in its simplest explanation, is the amount of pressure the ventricles need to contract against (workload) in order to empty the ventricles during systole. Thus any factors that increase or decrease the arterial pressures within the pulmonary and systemic circuits will increase or decrease the afterload, respectively (Ross 1976).

The heart rate is controlled by many different factors, of which many are not relevant to this thesis. However, those that are important are local and autonomic and catecholamine-related factors that alter the heart rate (Cobb 1994). Within the heart are atrial stretch receptors; and if the venous return is great enough to cause the atria to stretch beyond their normal limits, then the heart rate is increased to try to push the excess blood through the heart and into the arterial sides of the circuits. This effect is called the Bainbridge response and is well described in humans, but not yet in cats (Crystal & Salem 2012). Autonomic control is through a reflex loop which begins at the baroreceptors found within the aorta (aortic arch; afferent fibres of the vagal nerve) and carotid (carotid sinuses; afferent fibres of the glossopharyngeal nerve) arteries (Heesch 1999). These baroreceptors are stretch receptors and fire at a resting frequency. If the systemic blood pressure increases, then the firing rate of these baroreceptors increases and sends the signal to the brain, specifically the cardiovascular centre within the midbrain. Here, the signal of increased blood pressure is received and the cardiovascular centre sends descending signals to the heart to decrease the heart rate and inotropic capacity, to decrease the overall cardiac output and thus blood pressure. Vice versa, if the baroreceptors detect a decrease in blood pressure then their normal firing frequency will decrease and this signal is detected by the cardiovascular centre, which responds by increasing the sympathetic tone to the heart, which increases the heart rate and inotropic capacity to increase the cardiac output (Bertinieri et al. 1988). Furthermore, the adrenal glands are stimulated to release catecholamines (noradrenaline and adrenaline) which causes vasoconstriction and an increase in systemic vascular resistance which aims to restore the arterial blood pressure. If the heart rate is too high, there won't be enough time for ventricular filling and a decrease in arterial blood pressure will be detected because of the decrease in stroke volume and thus cardiac output.

The perfusion pressure of organs (e.g. brain, renal and liver) can be calculated by subtracting the internal organ pressure from MAP. Maintaining a normal perfusion pressure ensures that the blood accomplishes its function of transporting oxygen and nutrients to the organ and removing metabolic by-

products. Within the brain, the internal organ pressure is strongly related to the intracranial pressure. Therefore cerebral perfusion pressure is calculated by subtracting the intracranial pressure from MAP. The perfusion pressure is the pressure gradient and is used within the reapplied form of the Ohm's Law electrical equation to help determine the blood flow within the organ (Brown 2012). Vital organs, like the brain and kidneys, have an autoregulation function whereby the blood flow through the organ will be constant and within normal limits provided the MAP is within normal ranges. If MAP decreases below the lower limits of the autoregulation range for the organ then compensatory responses (vasoconstriction causing an increase in vascular resistance) occur within the organ blood vessels to try and maintain the perfusion pressure. However, considering the blood flow equation (blood flow to an organ = prefusion pressure/ vascular resistance) the blood flow might be decreased. This relationship holds true for most organs, except the heart. The heart receives perfusion through the coronary blood vessels (Siegel & Downing 1970). The left ventricle generates its perfusion pressure from the difference between the systemic diastolic arterial blood pressure and the filling pressure of the left atrium (left ventricular end-diastolic pressure). The right ventricle generates its perfusion pressure from the difference between the systemic systolic arterial pressure and right ventricular pressure. Therefore, the left ventricle only receives perfusion during diastole, while the right ventricle receives perfusion during diastole and systole. The fluctuating pressure gradient derived from filling and emptying of the atrium and changes in arterial pressure does not alter the coronary blood flow and this has been called coronary autoregulation. Also, when oxygen demand from the myocardium increases then the coronary blood flow increases, provided the arterial pressure is high enough, and this is called metabolic adaption (van de Hoef et al. 2012).

1.2.1.2 Physiology of the respiratory system

The respiratory system has a principal function of gas exchange, and thus, gases must move in and out the lungs to allow for this to take place. The minute volume is the total amount of gas that moves into and out of the lungs per minute and can be calculated by the following equation (Dugdale 2007):

$$\text{Minute volume} = \text{Tidal volume x Respiratory rate}$$

The respiratory rate is the number of breaths taken per minute and can be easily measured. The tidal volume is the volume of gas moving in (inspiratory tidal volume) and out (expiratory tidal volume) per breath and can be measured using various types of spirometers. In a healthy animal, the stimulation to breathe is triggered by the amount of carbon dioxide in the blood; and the normal arterial partial pressure of carbon dioxide ($PaCO_2$) reference interval in cats is 27-37 mmHg (Herbert & Mitchell 1971; Middleton et al. 1981). If the $PaCO_2$ increases above this range, because of issues related to the respiratory system, then the animal is classed as hypoventilating; vice versa, if the $PaCO_2$ drops below the lower reference interval than the animal is said to be hyperventilating (Hopper & Powell 2013). Oxygen can become a stimulus for ventilation when the arterial partial pressure of oxygen (PaO_2) drops lower than 50 mmHg; then the animal begins to breathe because of hypoxic drive and not because of changes in the $PaCO_2$. Hypoxaemia has been defined in many ways, but the most common definition is when the PaO_2 is less than the expected reference interval for the altitude at which the animal lives; at sea level, this is defined as a PaO_2 of less than 80 mmHg (Haskins 2015). However, clinically relevant hypoxaemia has been defined as a PaO_2 of less than 60 mmHg, regardless of altitude (Hopper & Powell 2013). The change in gas tensions is detected by chemoreceptors and can result in a change in minute volume (Dempsey & Smith 2013). The peripheral chemoreceptors are found in the aortic and carotid bodies and detect changes in $PaCO_2$ and PaO_2 (Dempsey & Smith 2013). The central chemoreceptors are located in the ventrolateral surface of the medulla oblongata and detect changes in pH within the cerebrospinal fluid mainly (CO_2 combines with water to produce carbonic acid, which dissociates into bicarbonate and hydrogen ions) (Harper 1996). In cats, is appears that the principal chemoreceptors that control minute volume are the central chemoreceptors and only minor input from peripheral chemoreceptors (Borison et al. 1980).

The respiratory system of cats is divided into two different functional regions; the conducting airways which are responsible for transferring gases in and out of the lungs, and the lower airways which are mainly responsible for gas exchange. The parts of the airways that do not participate in gas exchange are called dead space and the total amount of dead space within the airways can change (Robertson 2015). Physiological dead space is calculated by adding the anatomical dead space (which does not change and is considered the volume of all of the conducting airways until the terminal bronchi) and the alveolar dead space (which can change and it is where alveoli are ventilated but not perfused and therefore cannot participate in gas exchange). The physiological dead space can be applied to the tidal

volume to estimate how much gas enters the alveoli to participate in gas exchange. Furthermore, for gas exchange to occur, the alveoli need to be perfused with blood at the alveolar-capillary junction. The amount of ventilation to the amount of perfusion within the gas exchange regions of the lungs is termed ventilation to perfusion matching (V/Q matching). On the one extreme there is maximum ventilation but no perfusion, which is dead space ventilation; and on the other extreme end of the V/Q matching is intrapulmonary shunting where there is no ventilation but maximal perfusion (Petersson & Glenny 2014), which is shunt perfusion. Effective gas exchange occurs when the V/Q matching ratio of 0.8. Despite being able to measure this level of matching precisely, it is outside the scope of the work addressed in this thesis. However, oxygenation and ventilation indices have been used to identify, but not quantify, dead space ventilation or intrapulmonary shunting of blood and can be easily used in clinical practice to get an idea of how well the ventilation and perfusion systems are matching (Wagner 2015). Oxygen and carbon dioxide are the most important gases that are exchanged at the alveoli-capillary junction (Sarkar 2017). Oxygen is important for aerobic cellular metabolism and carbon dioxide is one of the by-products of cellular metabolism and blood is the transporter of these gases within the body. Frequently used oxygenation and ventilation indices are illustrated in **Table 1.1**.

Table 1.1 Equations used to calculate the oxygenation and ventilation indices in clinical practice. Normal mammalian ranges used to interpret the indices can be used in cats.

Name	Equation	Normal value range
Oxygenation indices		
P:F ratio; or arterial oxygen tension to fractional inspired oxygen ratio	Ratio = PaO_2/FiO_2	>300
Respiratory index	Index = $P(A\text{-}a)O_2/PaO_2$	<1.0
Alveolar-arterial oxygen tension gradient	$P(A\text{-}a)O_2 = PAO_2 - PaO_2$ where $PAO_2 = FiO_2(Pbar\text{-}PH_2O) - PaCO_2/RQ$	<10 mmHg (FiO_2 = .21) <100 mmHg (FiO_2 = 1.00)
Arterial-alveolar oxygen tension ratio	Ratio = PaO_2/PAO_2	0.8-0.9
Ventilation indices		
Dead-space to tidal volume ratio	Vd/Vt = $(PE´CO_2 - PaCO_2)/PaCO2$	20-40%
Arterial to end-tidal carbon dioxide tension gradient	$P(a\text{-}E´)CO_2 = PaCO_2 - PE´CO_2$	<5 mmHg

PaO_2: arterial tension of oxygen; PAO_2: alveolar tension of oxygen; FiO_2: fractional inspiration of oxygen; $P(A\text{-}a)O_2$: alveolar to arterial oxygen tension gradient; Pbar: barometric pressure; PH_2O: tension of water vapor; $PaCO_2$: arterial carbon dioxide tension; RQ: respiratory quotient (used a value of 0.8); Vd/Vt: dead-space to tidal volume ratio; $PE´CO_2$: end-tidal carbon dioxide tension; $PaCO_2$: arterial carbon dioxide tension; $P(a\text{-}E´)CO_2$: arterial to end-tidal carbon dioxide tension gradient

The alveolar to arterial oxygen tension gradient [$P(A\text{-}a)O_2$] and arterial to end-tidal carbon dioxide tension gradient [$P(a\text{-}E´)CO_2$] are the most commonly used and can reliably identify a failure of the

respiratory system to integrate adequately with the cardiovascular system (Armstrong et al. 2007; Yamauchi et al. 2011). These gradients determine how well the two gases are diffusing between the alveoli and the capillary bed. However, to identify the potential aetiology of the gas diffusion derangement, often more than one of these indices needs to be calculated and interpreted along with physiological data, like tidal volumes and dead space, for example (Zeiler et al. 2015).

1.2.1.3 Physiology of blood

Blood is continuously pumped through the blood vessels by the heart and it interacts with metabolising cells or alveoli at capillary beds. Capillary beds are regions where the blood is within one cell thickness (endothelial cells make up the capillary wall or membrane) of communicating with the interstitial fluid (fluid between tissue cells) and through various active and passive processes allows the exchange of gases, nutrients and by-products of metabolism (Cobb 1994). Therefore, blood could be thought of as the major transport system of the body. Blood is made up of a fluid component and a cellular component, and the exchanges that take place are mainly through the fluid component of the blood (Rudloff 2015). Plasma, the fluid component, contains dissolved gases (O_2 and CO_2) in water and electrolytes, proteins (albumin and globulins), biochemical substrates related to organ function and metabolism (glucose, urea, creatinine etc.), hormones and other signalling mediators (complement, pro- and anti-coagulation factors, pro- and anti-inflammatory mediators, cytokines etc.).

Water, the most abundant component of plasma, is essential to blood, but water moves freely throughout the entire body via different forces. The distribution of water throughout the body is important to understand in the context of this thesis. The amount of water within an adult cat body is approximately 60% of its body mass (Goggs et al. 2008). Most of the water is within the cells (intracellular compartment) and is termed the intracellular water (approximately 67% of total body water); the remaining water is outside the cells and is termed extracellular water (33% of total body water). The extracellular water is divided into two compartments, the interstitial compartment (25% of total body water) that bathes the tissue cells and the intravascular compartment (8% of total body water) which is the plasma (Broadstone 1999). Water moves freely across all tissue barriers and mechanisms exist to keep the water within respective compartments. The regulation of water movement between the

intravascular and interstitial compartment is via Starling's forces, and its movement between the interstitial compartment and the intracellular compartment via osmotic forces (Broadstone 1999; Mazzaferro & Powell 2013).

The classic Starling's forces include hydrostatic forces (influenced by the arterial blood pressure) and oncotic forces (influenced by proteins creating a colloidal osmotic force). The term force is used because the pressure is applied to a surface area (the capillary wall). However, to explain the Starling's forces, only mention of the pressure gradients and not the surface area to which it is applied is mentioned in this discussion. As blood moves towards the capillary beds, it must move through the precapillary arterioles which cause a decrease in the arterial blood pressure to approximately 40 mmHg just before entering the capillary bed (Stewart 2015). After traversing the capillary bed, the blood pressure drops and reaches approximately 16 mmHg as it leaves the capillary bed and enters the venules. During the transition through the capillary bed, the content of proteins remains the same, and therefore the oncotic pressure within the intravascular compartment remains the same (Rudloff 2015; Stewart 2015). The major contributor of the oncotic force during health within the intravascular compartment is albumin (80%), while the remainder is from circulating globulins (20%). Albumin is not only found within the intravascular compartment (30% to 40%), but also within the interstitial and intracellular compartments. The oncotic pressure (also termed colloidal osmotic force) draws fluid from the interstitial compartment into the intravascular compartment at roughly 25 mmHg. Therefore, the net pressure gradient of the Starling's forces at the arteriolar side of the capillary is 15 mmHg (40 mmHg – 25 mmHg) pushing fluid out of the intravascular compartment and into the interstitial compartment (Stewart 2015). At the venule side of the capillary bed, the net pressure gradient is -9 mmHg (16 mmHg – 25 mmHg) which means that fluid moves from the interstitial compartment back into the intravascular compartment (Sadava et al. 2006). Therefore, in health, the overall pressure gradient (15 mmHg – 9 mmHg = 6 mmHg) favours water to move from the intravascular compartment into the interstitial compartment. This water that is continually moving into the interstitial compartment at the capillary beds helps to maintain an osmotic gradient, which is important for the movement of water from the interstitial compartment into the intracellular compartment (Sadava et al. 2006). The excess water within the interstitial compartment is removed by the lymphatic system and eventually drains back into the intravascular compartment via the thoracic duct.

Osmotic force is also a pressure that is applied to a surface area (cellular membranes) and the pressure is termed the osmotic pressure. Osmotic pressure is a measure of the tendency of a solution to take in water via a process called osmosis (Rudloff 2015). Osmosis is the free movement of water across a selectively permeable membrane (cellular membranes, for example) from a dilute solution (hypoosmotic solution) to a concentrated solution (hyperosmotic solution) (Goggs et al. 2008). Furthermore, the amount of solute dissolved in a solution represents its tonicity, the more solutes dissolved within the water the higher its tonicity. With reference to the tonicity of plasma, intravenous fluids with high tonicity are termed hypertonic (hyperosmotic) solutions; whereas intravenous fluids with few solutes dissolved in them then they are termed hypotonic (hypoosmotic) solutions. The osmotic forces between the intravascular and interstitial compartments are generated by similar solutes and therefore considered to be the same and thus, movement of water is dependent on Starling's forces (Stewart 2015). The osmotic forces between the interstitial and the intracellular compartments are generated through different solutes and therefore an osmotic force could be experienced between these compartments. The effect of different solutes applying their forces across the cell membrane, especially if they are ions, is called the Gibbs-Donnan effect (Nguyen & Kurtz 2006). However, in health where the total water balance is normal, the forces generated by the different solutes are similar and therefore there is no major osmotic force existent between these two compartments. Thus the osmotic forces across all compartments are similar during health and any loss or gain of water or electrolytes must initially occur in the extracellular fluid (intravascular or interstitial) compartment (Goggs et al. 2008). If a change in the extracellular fluids water or electrolyte concentration occurs, then there will be an osmotic force among compartments and it takes time for the body to correct the balance to normalise the osmotic forces. Because, in health, the osmotic forces are similar across the compartments, the intravascular compartment is used to measure the osmolality of the plasma, and thus the body. Osmolality is defined as the concentration of a solution expressed as the total number of solute particles per kilogram. The osmolality of plasma can be measured using an osmometer, or more typically it is calculated using the following equation (Goggs et al. 2008):

$$\text{Plasma osmolality} = 2(\text{sodium} + \text{potassium}) + \text{glucose} + \text{blood urea nitrogen}$$

where the sodium, potassium, glucose and blood urea nitrogen are all plasma concentrations (mmol L^{-1}).

In cats, the reference interval for plasma osmolality is between 308 and 335 mOsm kg^{-1}, and is reported to be higher than that of dogs (290 and 310 mOsm kg^{-1}), mainly because of higher sodium concentrations in cats (Goggs et al. 2008). This reference interval is used to define what is considered isosmotic, or isotonic and any value above this interval is termed hyperosmotic (hypertonic) and any value below this interval is termed hypoosmotic (hypotonic). These terms are used to describe the type of water loss from the body or to categorise resuscitation fluids that are administered into the intravascular compartment (described later in section '1.2.5 Fluids used to resuscitate absolute hypovolaemia').

The cellular component of blood is made up of erythrocytes (red blood cells), leukocytes (white blood cells) and thrombocytes (platelets). While all these cells are essential to health and wellbeing, within the context of this thesis, and controlled haemorrhage perhaps, the red blood cells are important to elaborate on here. Platelets will be discussed in '1.2.9 Haemostasis theory' section. Red blood cells contain haemoglobin, and haemoglobin can act either as an oxygen transporter or as a buffer (Malte & Lykkeboe 2018). The transition between acting as an oxygen transporter or as a buffer depends on the local environment gas partial pressures (Grant 1982). When red blood cells traverse the capillary beds alongside alveoli they are exposed to high oxygen partial pressures which stimulate haemoglobin to become an oxygen transporter, known as the Haldane effect (Christiansen et al. 1914). When red blood cells traverse tissue capillary beds, there is a higher carbon dioxide partial pressure and this stimulates haemoglobin to offload oxygen and act as a buffer or transporter of carbon dioxide, called the Bohr effect (Bohr et al. 1904). The ability to act as a buffer is incorporated into the most effective and well described buffer system, the bicarbonate buffer system (this system will be discussed further in '1.2.8 Acid-base theory of blood' section). The principal function of blood is transportation and oxygen is one of the most important elements that require transportation. Oxygen can be transported as a dissolved gas or bound to haemoglobin and the content of oxygen in the arterial blood (CaO_2) can be calculated using this equation (Dunn et al. 2016):

$$\mathrm{CaO_2} = (1.39 \times \mathrm{Hb} \times \mathrm{SaO_2}) + (0.003 \times \mathrm{PaO_2})$$

where 1.39 is the Hufner's constant and it describes how many ml of oxygen can bind to haemoglobin when fully saturated; in cats this has been determined to be close to 1.39 ml of oxygen per gram of haemoglobin (Herrmann & Haskins 2005); Hb is the haemoglobin concentration (g dL^{-1}); SaO2 is the

oxygen-haemoglobin saturation of arterial blood (as a decimal); 0.003 is the solubility coefficient of oxygen (Vol% $mmHg^{-1}$ which is ml oxygen per dL); and PaO_2 is the arterial partial pressure of oxygen (using the mmHg pressure scale).

This equation highlights the importance of haemoglobin as the oxygen transporter of the blood because 97% of the oxygen content is bound to haemoglobin and very little is dissolved within the blood. Oxygen is not a very soluble gas within the blood (water). According to Fick's equation, the amount of oxygen that can be taken up by the blood (VO_2) traversing the alveoli capillary bed within the pulmonary circuit can be calculated with the following equation (Boller & Boller 2015; Dunn et al. 2016):

$$\mathrm{VO_2} = \text{Cardiac output x } (\mathrm{CaO_2} - \mathrm{CvO_2})$$

Therefore, the amount of oxygen that enters the arterial system is heavily dependent on the haemoglobin concentration and the cardiac output and the arteriovenous different in oxygen content (CaO_2 – CvO_2). This equation has also been used to calculate the oxygen consumption of the body if applied to the systemic circulation (Cooper 2018). The delivery of oxygen (DO_2) to the metabolising tissues is dependent on the arterial content of oxygen (CaO_2) and the cardiac output (DO_2 = cardiac output x CaO_2). It is paramount to ensure adequate delivery of oxygen to the metabolising tissues to maintain homeostasis and to prevent oxygen debt. In health, the oxygen consumption is supply-independent and therefore the animal does not reach an anaerobic threshold. However, if the supply begins to decrease below the oxygen consumption, then the oxygen delivery becomes supply dependent and an oxygen debt will begin to accumulate. Oxygen debt can be created when the supply is decreased, or if the oxygen consumption is too high (pyrexia for example), or a combination of both. The oxygen extraction (O_2ER) can be calculated using the following equation (Dunn et al. 2016):

$$\mathrm{O_2}ER = \frac{\mathrm{CaO_2} - \mathrm{CvO_2}}{\mathrm{CaO_2}}\ x\ 100\%$$

The normal oxygen extraction in most mammals is less than 30% when at rest and is thought to be similar in cats (Dunn et al. 2016). Therefore, this equation can be used to estimate if the animal is consuming enough oxygen for its metabolic demand. However, for proficient oxygenation of the metabolising tissues, the erythrocyte must be pliable in order to traverse the capillary bed. If the erythrocytes swell, such as when inflammatory mediators are circulating in the blood, then they are not pliable enough and can obstruct the capillary beds. If the capillary beds are obstructed then this will

result in a decrease of blood flow through the microcirculation and a decrease in tissue oxygenation (Scharte & Fink 2003).

1.2.2 Haemorrhage models

Animals have been used as medical research models for decades (Majde 2003). The main purpose of these models, for human medicine, has been focused on pre-clinical experimentation of potential therapies or to understand the pathophysiology of a process, such as haemorrhagic shock (Deitch 1998; Fulop et al. 2013). Unfortunately, many of these animal model-based preclinical studies, especially focusing on shock, do not translate into effective therapies for humans (Deitch 1998). However, in veterinary medicine, species-specific animal-based models do provide an insight into pathophysiology and potential therapies whereby bench-based outcomes can be utilised with confidence in clinical small animal practice, at least in dogs (Tait & Larson 1991; Friedman et al. 2003; Driessen et al. 2006; Barros et al. 2011; McBride et al. 2017). There are three different types of animal haemorrhage models that have been described over the past few decades: 1) fixed-volume haemorrhage, 2) fixed-pressure haemorrhage, and 3) uncontrolled haemorrhage (Deitch 1998; Lomas-Niera et al. 2005; Moochhala et al. 2009; Fulop et al. 2013).

Fixed-volume haemorrhage models involve removal of a predetermined amount of blood from the intravascular compartment over a predetermined time (Lomas-Niera et al. 2005). Usually, the systemic arterial blood pressure is monitored to elucidate the haemodynamic response to a specific volume of blood loss. However, some study designs have an initial planned volume to be removed to achieve and maintain a targeted systemic arterial blood pressure before administering resuscitation fluids (McBride et al. 2017). The volume of blood loss is based on the animal's estimated or calculated total blood volume wherein a percentage of this total circulating volume is removed. The range of blood withdrawn is between 20 and 60% of the total circulating blood volume (Moochhala et al. 2009). However, calculating the total circulating blood volume is not an easy task (Erlt et al. 2007), and therefore, the total blood loss is rather calculated on a per kilogram of body mass instead. Commonly used volumes range from 30 to 45 ml kg^{-1}, depending on the species of animal used (Moochhala et al. 2009). In dogs, experimental haemorrhage alone or with subsequent fluid resuscitation designs using the fixed-volume

approach remove 30 to 48 ml kg^{-1} over a time ranging from 11 to 100 minutes in conscious dogs (Carroll et al. 1988; Waisman et al. 1993) or 30 minutes in anaesthetised dogs (Driessen et al. 2006; McBride et al. 2017). In the anaesthetised dog studies, the blood was collected either from the femoral artery or the central vein, at a controlled rate 1.1 to 1.6 ml kg^{-1} per minute, depending on the study (Driessen et al. 2006; McBride et al. 2017). After the haemorrhage, the dogs were either left hypotensive for 60 minutes before fluid resuscitation (Driessen et al. 2006), or resuscitation fluids were administered immediately (McBride et al. 2017). In all studies, the dogs were euthanised humanely while under general anaesthesia (Driessen et al. 2006; McBride et al. 2017) or sedated with diazepam (benzodiazepine agonist) during conscious haemorrhage and allowed to die as a result of haemorrhagic shock (Carroll et al. 1988; Waisman et al. 1993). Overall, the advantage of this model is that physiological responses to acute haemorrhage can be measured and it allows the assessment of compensatory responses. The potential limitations of this model are that there is no induced trauma and that anaesthesia is required in some species (rats, mice and primates), therefore the outcomes do not easily translate into clinical practice (Lomas-Niera et al. 2005). We speculate that the cats will not tolerate conscious haemorrhage until they become hypotensive and therefore we would also have to conduct our research while the cats are under general anaesthesia. Another major disadvantage is that the degree of hypotension is uncertain, especially if large volumes of blood are being removed. Dogs have been reported to die because of extreme hypotension when more than 40% of their circulating blood volume was removed (Carroll et al. 1988; Waisman et al. 1993); we speculate that the outcome might be similar in cats. In humans, a haemorrhage exceeding 40% of the circulating volume, results in a mortality rate of more than 30% in clinical practice (Frankel et al. 2007). Therefore, most studies are designed to simulate this amount of haemorrhage in an attempt to describe the compensatory processes, define pathophysiological processes and the response to various therapies, like fluid resuscitation (Fulop et al. 2013).

In the fixed-pressure haemorrhage models the animal is haemorrhaged until a predetermined target systemic arterial blood pressure achieved; and then the animal is intermittently haemorrhaged to maintain this set pressure for the desired predetermined length of time (Lomas-Niera et al. 2005). This model has also been called the Wiggers method despite Penfield first describing it in 1919. The model has been used commonly in dog haemorrhage followed by resuscitation research (Penfield 1919; Wiggers 1942; Tait & Larson 1991; Friedman et al. 2003; Barros et al. 2011; Fulop et al. 2013).

Generally, the targeted pressure is where hypotensive shock can result and has been defined as a MAP ranging from 20 and 55 mmHg, depending on the species and study objectives (Fulop et al. 2013). The targeted pressure, once achieved, is maintained for a predetermine time which ranges from as short as 15 minutes up to 5 hours (Lomas-Niera et al. 2005; Moochhala et al. 2009; Fulop et al. 2013). Furthermore, no consensus definition of hypotension exists in small animal practice, but in routine practice, anaesthetised dogs are considered to be hypotensive if their MAP is < 62 mmHg (Ruffato et al. 2015). We speculate that similar minimum arterial pressure cut-offs are applicable for defining hypotension in cats. For the purposes of this thesis we define hypotension as a MAP of less than 60 mmHg, and severe hypotension as a MAP of less than 40 mmHg (Wagner & Brodbelt 1997). In fixed-pressure models, dogs undergo haemorrhage until a target MAP of 35 to 50 mmHg is reached and then usually maintained for 45 to 90 minutes before fluid resuscitation (Tait & Larson 1991; Friedman et al. 2003; Barros et al. 2011). The resuscitation fluids were administered at fixed ratios to the volume of blood lost over 15 minutes (Barros et al. 2011), or until the MAP returned to a targeted normotensive pressure (Friedman et al. 2003), or at a fixed rate until the study ended (Tait & Larson 1991). In all studies, the dogs were anaesthetised during the experimental procedures and were humanely euthanised at the end of the study. The advantage of this model is that the extent and duration of hypotension is controlled. The level of experimental standardisation and reproducibility using this model is said to be higher compared to the fixed-volume haemorrhage model (Fulop et al. 2013). Therefore, this model has been used to investigate the pathophysiology and changes in organ function and tissue injury during haemorrhagic shock (Fulop et al. 2013). However, this model is the least clinically comparable model because the compensatory mechanisms are continually suppressed in order to maintain the targeted arterial blood pressure. Also, animals are usually anaesthetised during the investigative procedures, which could affect the translation of bench studies to clinical practice (Lomas-Niera et al. 2005; Fulop et al. 2013).

The fixed-volume and fixed-pressure models are easily reproducible and the outcomes of various haemorrhage and resuscitation type studies have yielded closely comparable results (Fulop et al. 2013). However, the outcome of these models, even if they are species-specific, rarely translate into effective clinical practice therapies and recommendations because there are too many variables that are controlled (Lomas-Niera et al. 2005; Moochhala et al. 2009; Fulop et al. 2013).

A third model, the uncontrolled haemorrhage model, has been described, aiming to emulate clinical case presentations. In this model, the only aspect that is standardised is the type and the site of vascular trauma; this could include crushing or lacerating the liver or spleen, transecting or puncturing a large artery, or amputating an appendage (Lomas-Niera et al. 2005; Moochhala et al. 2009; Fulop et al. 2013). Because of individual responses to vascular insult, the experimental control cannot be precisely achieved. This lack of controlling of the study variables makes this model the most clinically relevant compared to the fixed-volume and fixed-pressure models (Lomas-Niera et al. 2005; Moochhala et al. 2009; Fulop et al. 2013). It allows for better preclinical investigations where animal models are used to define, predict and investigate physiology and pathophysiology of haemorrhage-induced hypotension and haemorrhagic shock in humans. The addition of some form of trauma activates many other homeostatic pathways and cascades which affect the physiological outcome compared to simple uncontrolled haemorrhage. This model has been used extensively in translational based-investigations and has hardly been reported in the veterinary literature. Instead, dogs and cats that present to private and academic hospitals with trauma-associated injuries and shock have been used in observational, retrospective fluid resuscitation studies (Silverstein et al. 2012; Giudice et al. 2018). However, as with the uncontrolled haemorrhage model, there are many variables that cannot be controlled, such as the time between injury and the administration of resuscitation fluids, the extent of the injury and when the injury involves multiple organ systems.

Regardless of the model selected, the outcome of many of these studies is descriptive and mostly focused on haemodynamic data (Majde 2003). Most of the emphasis has been based on replacement fluid therapies to treat massive blood loss, and hardly any attention is focused on the broad animal response. To comprehensively understand the effects of haemorrhage and fluid resuscitation then the study must be designed to capture more variables than previous reports only focusing on haemodynamic variables. Comprehensive studies capturing many variables allows for outcomes that can be translated into clinical practice. Also, many of the controlled variables tend to prevent the translation of bench findings to clinical practice. The investigators need therefore to not only be aware of these variables, but also to try to align them with real clinical scenarios to allow for effective translation of findings and meaningful recommendations to be made.

1.2.3 The physiology of haemorrhage in cats

Cats have been used as haemorrhage models to study the effects of haemorrhage on many different organs, since as early as 1921 (Doi 1921). There was then a hiatus for a number of decades until renewed interest in haemorrhage and haemorrhagic shock research surged in the 1960s mainly, but continued until the early 1990s (Groom et al. 1965; Thrivikraman et al. 1993). Cats are not included in the last two decades of reviews focusing on animal-based haemorrhage models (Lomas-Niera et al. 2005; Moochhala et al. 2009; Fulop et al. 2013). This omission is because they do not make good translational models for human medicine due to their smaller circulating blood volume and physiological peculations that will be discussed in this section. As a result, little research has been conducted from the 1990s onwards, especially with regards to haemorrhage and fluid resuscitation. Scant reports on fluid resuscitation can be found, mostly on trauma cats presenting to private and academic hospitals. These studies will be discussed in the '1.2.5 Fluids used to resuscitate absolute hypovolaemia' section of the literature review.

There does not seem to be a consensus statement defining severe haemorrhage. From examining the literature, two important criteria used are to define severe haemorrhage 1) the volume of blood lost compared to the total circulating volume, and 2) the rate at which the blood is lost (Majde 2003). Many of the dog haemorrhage model studies determine the survival of the animals to acute blood loss (<30 minutes), and all report that the dogs survived when up to 40% of their circulating blood volume was removed (Driessen et al. 2006; Barros et al. 2011; McBride et al. 2017); despite some dogs experiencing severe hypotension (Carroll et al. 1988; Waisman et al. 1993). However, deaths in conscious dogs have been reported when 45% of the circulating blood volume had been removed acutely without resuscitation; if 50% or more is removed then it can be 100% fatal (Carroll et al. 1988). This observation in dogs may be similar in cats and therefore we will define severe haemorrhage as a loss of 40% of the circulating blood volume. The total circulating blood volume in cats is smaller than in dogs (Rozanski & Rondeau 2002). Based on previous investigations, the measured circulating whole blood volume of cats ranges from 52.6 ± 6.8 to 59.6 ± 5.8 mL kg^{-1} (Groom et al. 1965; Mott 1968).

Hypovolaemia means that there is too little volume of fluids within the intravascular compartment. Two mechanisms causing hypovolaemia have been described. The first mechanism is a direct loss of

volume through haemorrhage or excessive insensible fluid loss such as by polyuria (increased volume of urination compared to normal), fluid sequestering into the stomach and diarrhoea, for example. This direct loss of volume from the intravascular compartment is termed an absolute hypovolaemia. The second mechanism is an indirect loss whereby the intravascular fluid volume is not lost, but rather the anatomical volume of the blood vessels expand through vasodilation, which causes the intravascular volume to be relatively less compared to a normal blood vessel tone. This is termed a relative hypovolaemia. Drugs and other mechanisms, such as bee stings, can cause excessive histamine release which causes systemic widespread vasodilation, thus resulting in relative hypovolaemia and associated hypotension. Some of the drugs used for anaesthesia or analgesia can cause vasodilation and therefore we have selected drugs that have a minimal effect on blood vessel tone. These drugs are discussed later in '1.2.4 Anaesthetic and analgesic drug effects on cats' section.

The physiological effects of haemorrhage on various organ systems have been studied in cats and include the cardiovascular system (Doi 1921; Kenney & Neil 1951; Groom et al. 1962; Groom et al. 1965; Greenway & Lawson 1966; Mott 1968), respiratory system (Doi 1921; D'Silva et al. 1966; Miserocchi & Quinn 1980), central nervous system (Thrivikraman et al. 1988; Kovach et al. 1991; de Witt et al. 1992; Thrivikraman et al. 1993), endocrine and metabolic systems (Clark & Silva 1967; Andersson et al. 1982; Lautt et al. 1982; Bereiter & Gann 1986; Bereiter et al. 1986a, b; Gaumann & Yaksh 1988) and the gastrointestinal tract and other splanchnic organs (Barcroft et al. 1925; Barcroft & Stephens 1927; Baker & Mendel 1967; Greenway et al. 1967; Greenway & Stark 1969; Greenway & Lister 1974; Lautt et al. 1980; Breznock & Strack 1982).

In chloralose alone or in combination with urethane, or urethane alone anaesthetised cats, the cardiovascular system responds in a classic mammalian fashion whereby the drop in arterial blood pressure during haemorrhage was accompanied with an increase in heart rate (Doi 1921; Groom et al. 1965). However, counterintuitively, in a haemorrhage study by Mott (1968) in sodium pentobarbitone anaesthetised cats, the heart rate in two of the six cats fell progressively. Also, predictably, the stroke volume decreased with the drop in blood pressure (Doi 1921). The cats increased their systemic vascular resistance in an attempt to maintain normotensive arterial blood pressures after a blood loss of approximately 20% of the circulating blood volume (Groom et al. 1965). The trigger for the increase in systemic vascular resistance appears to originate in the carotid artery baroreceptors; with the response being immediate and maintaining normal cardiac output in the initial phase of haemorrhage

(Groom et al. 1962). Also, in sodium pentobarbital or chloralosed anaesthetised cats, the aortic body chemoreceptors, and not the vagal nerve alone, influence blood pressure during haemorrhage and their activation (through decreased oxygen levels) can accelerate a drop in blood pressure during fixed-pressure controlled (30 to 70 mmHg arterial blood pressure) haemorrhage (Kenney & Neil 1951). The degree of vagal nerve activity during haemorrhage can be decreased by increasing the FiO_2 (Kenney & Neil 1951). Furthermore, the vagal nerve and these chemoreceptors may cause apnoea which is thought to be the leading cause of death in haemorrhaging and hypotensive dogs and cats (Kenney & Neil 1951). Venous return, which is an important determinant of stroke volume and thus cardiac output, was decreased after haemorrhage in pentobarbitone anaesthetised cats (Greenway & Lawson 1966). Importantly, the increase in systemic vascular resistance was different in the different organs and regions being studied. Initially, the vascular resistance was increased in the kidneys and pelvic limbs and not in the cranial vena cava (head) and the liver (Greenway & Lawson 1966). Normotension was maintained when 8 to 27 ml kg^{-1} of blood was removed before the cats became hypotensive (Groom et al. 1965). The amount of blood loss required before the onset of hypotension has been reported to be highly variable in cats, even in those of similar weight (Groom et al. 1965). Despite the high variability in blood volume that must be lost, Groom et al. (1965) found that arterial blood pressure will be maintained if the residual circulating blood volume after haemorrhage is maintained above 41 ml kg^{-1}. However, once the state of critical hypotension was achieved (40 to 60 mmHg), as defined by Groom et al. 1965, then the heart rate began to drop and the cats quickly succumb to absolute hypovolaemia (Groom et al. 1965). These studies were informative and helped us create a study design, however, the haemorrhage was intermittent and done by removing small increments of blood at set time intervals over several hours (Doi 1921; Groom et al. 1965, Greenway & Lawson 1966; Mott 1968). This study design does not translate well in cats that sustain a severe acute haemorrhage, as it often is the case in clinical practice.

The respiratory system of anaesthetised cats tends to respond in a uniform and predictable manner (Doi 1921; D'Silva et al. 1966; Miserocchi & Quinn 1980). As stated by Doi (1921) based on his study, "It is well known that when an animal loses blood, respiratory frequency increases while tidal volume gets smaller". He found that as the blood pressure decreases, the amount of oxygen taken up by the blood also decreases. Also, the blood flow through the lungs decreases as haemorrhage progresses (Doi 1921). If the arterial blood pressure is lowered from 100 mmHg to 60 mmHg through haemorrhage

in spontaneously breathing pentobarbital anaesthetised cats, then a rapid drop in the end-tidal carbon dioxide ($P_E'CO_2$) and an increase in arterial pH and minute volume (resulting mostly from an increase in tidal volume) occurs (D'Silva & Mendel 1966). The drop in $P_E'CO_2$ occurs because of a change in minute volume and not because of reduced pulmonary blood flow, which leads to uneven alveoli perfusion and an increase in physiological dead space, as described in ventilated dogs (D'Silva & Mendel 1966). Furthermore, the authors found that the control of minute volume during haemorrhage was by the carotid body chemoreceptors, and that the resultant increase in minute volume causes a respiratory alkalaemia (D'Silva & Mendel 1966). A study of the ventilatory response to acute blood loss in pentobarbital anaesthetised cats under hyperoxic (fraction of inspired oxygen: FiO_2 = 0.5) iso-capnoeic conditions compared the effects of changes in ventilation to the change (drop) in arterial blood pressure and not to the volume of blood drawn (Miserocchi & Quinn 1980). Overall, as blood pressure dropped from approximately 150 mmHg to about 70 mmHg (assumed to be systolic arterial blood pressure readings), the minute ventilation increased by 2.5-fold. The increase in minute ventilation occurred in two distinct phases. An initial phase, where the blood pressure dropped from approximately 150 mmHg to around 100 mmHg, was characterised by an increase in the respiratory rate due to the excitatory effects on the bulbo-pontine timing of respiration rate; this effect was thought to result from the withdrawal of inhibitory afferents from the aortic baroreceptors (Miserocchi & Quinn 1980). The initial phase of the change in minute ventilation occurred when there was an increase in systemic vascular resistance (increase in vasomotor tone). A second phase was characterised by an increase in tidal volume (and inspiratory pressure); the stimulus originated from the carotid chemoreceptors with a minor effect from the decrease in inhibitory afferents from the carotid baroreceptors. The most likely stimulus to activate the carotid chemoreceptors during haemorrhage is as a result of two interlinked mechanisms. The first mechanism is the reduced blood flow past the glomus cells of the carotid sinus (Miserocchi & Quinn 1980). The second mechanism is the decreased oxygen uptake in the alveoli during reduced blood flow which leads to hypoxaemia, especially when the arterial blood pressure is less than 70 mmHg (Doi 1921).

The central nervous system has been studied in isoflurane or chloralose-urethane or pentobarbital anaesthetised cats undergoing mild (de Witt et al. 1992) or moderate haemorrhage and particular emphasis has been placed on determining the cerebrovascular reactivity (Kovach et al. 1991) and noradrenergic activity in relation to pituitary gland secretions (Thrivikraman et al. 1988; Thrivikraman et

al. 1993). In isoflurane-anaesthetised cats (FE′iso 1.6%; FiO_2 0.3), haemorrhage (approximately 30% total circulating blood volume; 18 ml kg^{-1} at 3 ml $minute^{-1}$) until the cats were hypotensive (MAP 50 mmHg) did not affect the cerebral blood flow or change electroencephalographic activity. However, if the cat sustained trauma (moderate fluid-percussion traumatic brain injury administered at 2.2 atmosphere pressure) and then underwent similar haemorrhage to induce hypotension, and then was administered a hydroxyethyl starch fluid (18 ml kg^{-1}; 10% hetastarch suspended in 0.9% sodium chloride; administered at 3 ml $minute^{-1}$) for resuscitation, it resulted in a decrease in cerebral blood flow, cerebral oxygen delivery and changes in electroencephalographic activity. To corroborate and identify the cause of the decreased cerebral blood flow, Kovach et al. (1991) found that after haemorrhage induced hypotension (50 mmHg), there is an impairment of cerebrovascular relaxation despite high concentrations of neuroreceptor agonists (acetylcholine, adenosine). They also found that the cerebrovascular constriction responds to 5-hydroxytryptamine and not noradrenaline or prostaglandin F2alpha (Kovach et al. 1991). Noradrenaline turnover within the locus coeruleus has been investigated during haemorrhage in cats (Thrivikraman et al. 1988; Thrivikraman et al. 1993). The locus coeruleus is a nucleus in the pons of the brainstem and is involved with responses to stress and anxiety. Furthermore, the locus coeruleus is also part of the reticular activating system and thus will receive many neuronal signal inputs from the body. The locus coeruleus and noradrenergic system are responsible for modulating the cortical, subcortical, cerebellar, brainstem and spinal cord neural circuits (Benarroch 2009). In chloralosed-urethane anaesthetised cats, which underwent a rapid (3 minutes) haemorrhage of 20% of their circulating volume, there was an increase in plasma adrenocorticotropic hormone (ACTH). The neurotransmitter noradrenaline also increased within 5 minutes after haemorrhage-induced hypotension (Thrivikraman et al. 1988). Furthermore, the increased noradrenaline release modulates the release of ACTH and vasopressin which, together are through to be responsible for the initiation of the systemic compensatory responses to acute haemorrhage (Thrivikraman et al. 1993).

The endocrine and metabolic response of cats to haemorrhage has been investigated well, with a particular interest on the hypothalamus-pituitary gland-adrenal glands axis (HPA axis). Chloralose-urethane anaesthetised cats that were haemorrhaged (30 to 46% circulating blood volume, bled within 1 minute) to reach at target mean arterial pressure of 60 and 40 mmHg had a rapid (within 15 minutes) and profound increase in arterial plasma concentration of adrenaline and noradrenaline (Andersson et

al. 1982). The release of these catecholamines also modulated the increased release of dopamine, glucose, cyclic adenosine monophosphate, glycerol and glucagon but a decreased release in insulin (Andersson et al. 1982). Furthermore, Bereiter et al. (1986a) found that the release of adrenaline evoked by haemorrhage (10%, 20% and 30% blood loss at 10% withdrawal minute^{-1}) was dependent on the rate of blood loss only when large volumes (30% circulating blood volume) were removed in chloralose-urethane anaesthetised cats. Noradrenaline release was not dependent on the rate of haemorrhage, but rather correlated ($r^2 = 0.689$; $p < 0.001$) with the volume of blood lost (Bereiter et al. 1986a). Also, glucose concentrations were proportional to the volume of blood lost and not affected by the rate of haemorrhage (Bereiter et al. 1986a). There is a priming effect on catecholamine (noradrenaline and adrenaline) release where an initial blood loss (20% of circulating blood volume) is followed by a subsequent blood loss within 90 minutes causing an increase in catecholamine release (Bereiter & Gann 1986). Chloralose-urethane anaesthetised cats that underwent haemorrhage (10 to 30% circulating blood volume) at a fast (10% blood volume minute^{-1}) or slow (2% blood volume minute^{-1}) rate showed that the cortisol concentration increases in proportion to the volume of blood lost only during fast haemorrhage and not during slow haemorrhage (Bereiter et al. 1986b). Bereiter et al. (1968b) also found that the rate and volume of blood loss determined the ACTH response where the recovery of the blood pressure after rapid haemorrhage may attenuate the increase in ACTH. Furthermore, there is an apparent dissociation between the ACTH and cortisol responses to slow haemorrhage after small volumes of blood being lost, but not after large (20 and 30% circulating blood volume) volumes (Bereiter et al. 1968b). The adrenal glands also release other substances, like neuropeptides, in response to haemorrhage (Gaumann & Yaksh 1988). Halothane (0.8% end-tidal halothane concentration) anaesthetised cats that underwent haemorrhage (approximately 40% circulating blood volume at a rate of 2 ml minute^{-1} using an electronic pump) had an increased release of some adrenal neuropeptides (methionine-enkephalin, neuropeptide Y, peptide YY, neurotensin) but not others (vasoactive intestinal polypeptide, cholecystokinin and bombesin), measured at the adrenal vein (Gaumann & Yaksh 1988). Along with catecholamine release during haemorrhage, vasopressin (a powerful vasoconstrictor) is also released when the arterial blood pressure is dropped (at least a 80 mmHg drop in blood pressure acutely within 20-30 seconds by removing blood from the femoral artery) during haemorrhage in chloralosed anaesthetised cats (Clark & Silva 1967). The adrenal glands and sympathetic innervation to the liver appear to be the main cause for the rapid hyperglycaemia (peaks

15 minutes after haemorrhage) that is seen during acute haemorrhage-induced (15.3 ml minute^{-1} of blood withdrawn until hypotensive using an electronic device) hypotension (MAP of 50 mmHg) in pentobarbital anaesthetised cats (Lautt et al. 1982). Also, nonadrenal hormones like growth hormone, glucagon, angiotensin and vasopressin play a minor role in creating a post-haemorrhage and hypotensive hyperglycaemia (Lautt et al. 1982).

The gastrointestinal tract (Baker & Mendel 1967) and other splanchnic organs (Greenway & Lister 1974), especially the liver (Greenway et al. 1967) and spleen (Barcroft et al. 1925; Barcroft & Stephens 1927; Greenway & Stark 1969; Lautt et al. 1980; Breznock & Strack 1982), have been investigated in haemorrhaged cats. Splanchnic nerves control the blood flow to the intestines. Stimulation of those nerves causes vasoconstriction. However, the cat intestine seems to have an “autoregulatory escape” whereby blood flow is preserved (Folkow et al. 1964a). This autoregulatory escape has been studied during sympathetic nerve stimulation and by noradrenaline infusions. The mechanism of this escape is thought to be by opening of submucosal shunts (Folkow et al. 1964b). However, during haemorrhage (blood volume removed until MAP was 50 mmHg and maintained for 4-5 minutes), cats did not develop the autoregulatory escape, resulting in decreased intestinal perfusion and blood flow as well as oxygen consumption (Baker & Mendel 1967). The decrease in oxygen consumption could be because of patchy-perfusion of the mucosa leading to areas being devoid of perfusion, or could result from the demand for oxygen consumption being determined by blood flow (Baker & Mendel 1967). Regardless, we speculate that poor blood flow to the intestinal tract could cause mucosal breakdown and complicate the management of the cat during hospitalisation. The splanchnic vasculature comprises capacitance vessels and thus has a reservoir function, where a large volume of the circulating blood is stored in low flow circuits. Greenway and Lister (1974) investigated the capacitance effects and blood reservoir function of the splanchnic vessels in pentobarbitone anaesthetised cats that underwent mild non-hypotensive haemorrhage (0.5 to 0.6 ml kg^{-1} per minute until 10 ml kg^{-1} of blood was withdrawn; 19% circulating volume) or transfusion (18 ml kg^{-1} which was 34% of circulating blood volume). They found that after small haemorrhage or modest transfusion, the splanchnic vessels can mobilise or store up to 65% of the volume of blood removed from or transfused into cats, respectively (Greenway & Lister 1974). The major mechanism responsible for the reflex regulation of the reservoir function seems to involve the atrial receptors and the sympathetic innervation of the splanchnic capacitance vessels (Greenway & Lister 1974). During the initial haemorrhage phase (approximately 4% of circulating blood

volume), blood is mobilised from the liver (≈21%) and gastrointestinal tract (≈22%), and as haemorrhage progresses (approximately 15% of circulating blood volume), then the spleen (≈19%) starts mobilising blood (Greenway & Lister 1974). Similarly, Lautt et al. (1980) reported that the liver mobilises a fixed proportion of the circulating volume (26 ±6%), regardless of the extent of blood loss during acute haemorrhage (15.3 ml minute^{-1} using an electronic pump until MAP was 50 mmHg) in pentobarbital or ketamine-chloralose anaesthetised cats. Furthermore, they found that denervation of the liver and administration of phentolamine, an alpha adrenoceptor antagonist, had no effect on the liver's capacity to mobilise blood (Lautt et al. 1980). Greenway et al. (1967) found that hepatic artery flow does not decrease until the arterial blood pressure drops below 80 mmHg (presumed to be systolic arterial blood pressure), but the portal vein blood flow drops profoundly during haemorrhage of pentobarbitone anaesthetised cats. They surmised that during haemorrhage there is vasodilation of the hepatic artery vascular bed and vasoconstriction of the vascular beds drained by the portal vein, which ensures that adequate oxygen supply to the liver is maintained as much as possible (Greenway et al. 1967). During haemorrhage (bled 10 ml of blood every 10 minutes) in urethane anaesthetised cats the spleen contracts and can discharge 14-28 ml of blood into the circulating blood pool (Barcroft et al. 1925; Barcroft & Stephens 1927). The response of the spleen to haemorrhage has been corroborated by others (Greenway & Stark 1969; Greenway & Lister 1974). Greenway and Stark (1969) further concluded that the splenic contraction and vasoconstriction are due to the activity of the splenic nerve and the adrenal medullae of the adrenal glands only, after rapid haemorrhage-induced (14 ml kg^{-1} per minute until MAP was 50 mmHg) hypotension. Breznock and Strack (1982) found that after a 6 ml kg^{-1} haemorrhage in awake cats that the circulating blood volume was at least 10 ml kg^{-1} more in cats with a spleen compared to splenectomised cats. This finding is similar to what Barcroft et al. (1925) and Barcroft and Stephens (1927) have reported, and clearly identifies the spleen as a reservoir of blood in that species.

Even though there has been decades of research into the effects of haemorrhage in cats, dogs and humans, there are still no reports of a strong relationship between the volume of blood being lost and the clinical signs that present during haemorrhage (Pacagnella et al. 2013). Of all of the variables assessed by Pacagnella et al. (2013), the shock index (systemic systolic arterial blood pressure divided by the heart rate) was the only one that demonstrated promise in human medicine; and this might be true for dogs too (McGowen et al. 2017) but it has not yet been evaluated in cats. Therefore, we still

rely on classic blood transfusion triggers; points at which the medical practitioner decides that the benefit of a blood transfusion outweighs the risk of the transfusion. The classic transfusion triggers in veterinary science are when a critical estimated volume of witnessed blood loss has occurred, such as during a surgical procedure; or when the haematocrit decreases below a trigger threshold, and this value is approximately 0.2 L L^{-1} in cats, depending on chronicity, physiological state and oxygen demand (Kirby 1995; Barfield & Adamantos 2011). However, these 'rules of thumb' have lasted for decades and have not, at least under surgical and anaesthesia conditions, been investigated. It is not known if there are better indicators to determine and quantify acute haemorrhage in cats, which could be used as physiological triggers to commence either volume resuscitation with fluids or with fresh whole blood. Such knowledge would improve our ability to predict the need for life-saving interventions (Pinsky 2015). It appears that the shock index is a possible useful variable, however, the blood pressure and heart rate can change because of anaesthetic drug effect or the surgical procedure and not just because of hypovolaemia.

1.2.4 Scoring systems used to assess acute haemorrhage

In human trauma medicine, many pre-hospital scoring systems can be used in the field, or in emergency rooms, which could help predict if the patient is suffering from massive haemorrhage (> 30% blood lost acutely) or to help predict the requirement of a massive blood transfusion (> 10 units of blood products administered within a few hours). Examples of these scoring systems include the trauma associated severe haemorrhage (TASH), revised assessment of bleeding and transfusion (RABT), emergency transfusion score (ETS), Larson score, Prince of Wales Hospital (PWH score)/Rainer score, and the shock index (SI) (Terceros-Almanza et al. 2019). The TASH score incorporates SAP, haemoglobin concentration, presence of intra-abdominal fluid, complex long bone and/or pelvic fractures, HR, base excess and sex as variables (Yucel et al. 2006). The TASH score can range from 0 to 28; the higher the score, the higher the probability of the patient requiring a massive blood transfusion. The RABT score is calculated from a 4-point score (Joseph et al. 2018). Points are allocated for a penetrating wound, a SI of ≥ 1, pelvic fractures or a positive focused assessment with sonography for trauma (FAST

scan). Hanna et al. (2020) found that the RABT score is a valid tool to predict the need for massive transfusion in severely injured trauma patients. The ETS score is calculated from arterial blood pressure, free fluid on ultrasound, clinical instability of the pelvic ring, age, admission from the scene and the trauma mechanism (Kuhne et al. 2008). This score is used to predict the blood transfusion requirements of severely injured patients. The Larson score (or Larson model) incorporates four variables where a point is allocated if the HR > 105 beats minute^{-1}, SAP < 110 mmHg, haemoglobin concentration < 11 g dL^{-1}, and base excess < -6 mmol L^{-1} (Larson et al. 2010). The PWH score (Rainer score) is determined from seven variables where a point is allocated if the SAP < 90 mmHg, Glasgow Coma Scale < 8, HR > 120 beats minute^{-1}, computer tomography scans or FAST scans are positive for free fluid, haemoglobin concentration < 7 g dL^{-1}, base deficit > 5 mmol L^{-1}, and the presence of displaced pelvic fractures (Rainer et al. 2011). A score of greater than 6 predicted the requirement of a massive blood transfusion accurately. Lastly, the SI, first described in 1967 (Allgower & Burri 1967) has already been investigated in veterinary medicine (McGowan et al. 2017) and mentioned in this thesis (section 'Chapter 3: Biomarkers that indicate an endpoint for administering lactated Ringer's solution or 6% tetrastarch 130/0.4 fluids to resuscitate haemorrhage-induced hypovolaemic anaesthetised cats: a concept of volume-endpoints to prevent iatrogenic fluid overload').

The scoring systems used during the peri-operative period in patients at high risk of severe acute haemorrhage are many but include the obstetric shock index, the universal definition of perioperative bleeding, European coronary artery bypass graft (E-CABG) bleeding severity score, bleeding severity scale, and the perioperative risk of blood transfusion (PORT), for example. The obstetric shock index is the SI which is applied during obstetric related surgical procedures during the peri-partum period (Le Bas et al. 2014). The universal definition of perioperative bleeding and the E-CABG bleeding severity score are similar in that they merely quantify how much blood (or blood produces) is administered to a patient during surgery. The higher the 'class' or 'grade' then the more blood is administered to the patient. These systems are used for predicting the outcome of surgical procedures (Bartoszko et al. 2018). The bleeding severity scale is a visual scoring system that surgeons can use to estimate the rate of blood loss visually. The scale is made up of 5 grades where grade 0 indicates no bleeding (< 1 mL minute^{-1}) and 4 indicates unidentified or inaccessible spurting or gushing of blood (> 50 mL minute^{-1}). The surgeon makes use of visual presentation, anatomic appearance and a qualitative description to decide on the grade of bleeding severity (Lewis et al. 2017). The PORT score is used to predict the risk

of requiring a blood transfusion in patients undergoing cardiac procedures, and there are seven variables which are used: age, sex, haemoglobin concentration, body surface area, EuroSCORE (European system for cardiac operative risk evaluation; www.euroscore.org), creatinine concentration, and type of operation to be performed (Klein et al. 2017).

All of the mentioned scoring systems are examples of what is currently being used in human medicine and there are many more available. The SI is the only human-based scoring system that has been used in veterinary trauma medicine (McGowen et al. 2017). There are no scoring systems used to quantify intraoperative blood loss during the peri-anaesthetic period. However, in veterinary trauma and critical care medicine, there are two commonly used scoring systems which could prompt the veterinarian to resuscitate the patient with fluids or blood products because of a suspicion of severe haemorrhage. These are the animal trauma triage (ATT) score and the acute patient physiological and laboratory evaluation (APPLE) score. In the ATT, the veterinarian is required to assign a grade (0 to 3) to each assessed organ system which includes perfusion, cardiac, respiratory, skeletal, neurological, and eye, muscle and integument systems (Rockar et al. 1994). Grading the perfusion, cardiac and respiratory systems could prompt a resuscitation effort that could include administering fluids, blood products, or both. The APPLE score has been described for cats and exists in two versions, one using 8 variables and the other using 5 variables. The 8-variable form predicted the outcome of the hospital stay better compared to the 5-variable form (Hayes et al. 2011). The 8-variable form made use of mentation score, temperature, MAP, lactate, packed cell volume, urea, chloride and body cavity fluid score (FAST scans or computer tomography).

The two major outcomes that are evident when examining these scoring systems is that 1) they make use of very similar variables despite having different cut off values, and 2) they incorporate more than one variable. Furthermore, the scoring systems are designed with a specific intention or purpose. They quantify haemorrhage, or they assess the risk for major transfusions, or they stratify patients into different risk categories, or they predict hospital stay outcome.

1.2.5 Anaesthetic and analgesic drug effects on cats

One of the major concerns, common to all of the haemorrhage research models and haemorrhage research, is the use of anaesthetic and analgesic drugs and the potential impact these drugs have on interpreting the data emanating from the research. Also, there is a major concern with extrapolating the findings and data to a conscious, or even a trauma patient. For our study, we wanted to emulate clinical practice and investigate haemorrhage and fluid resuscitation while cats are anaesthetised. Therefore, anaesthesia was not a major concern for the purposes of our study. However, to improve translation of our findings into clinical practice, we made use of a standardised drug combination known for its minimal cardiovascular and respiratory effects combined with good analgesic properties. Essentially, the standardised drug combination had to meet the requirements for balanced anaesthesia for cats undergoing elective or emergency surgery (Ilkiw 1999). Despite the drug's having reduced cardiovascular and respiratory effects, we cannot state with certainty what the drug effects on the anticipated compensatory responses, autonomic nervous system activity and the HPA-axis will be. We made use of buprenorphine as a pre-anaesthetic drug, alfaxalone as the induction drug to induce anaesthesia, and isoflurane mixed in oxygen to maintain the anaesthesia for the duration of the study procedures. We also administered meloxicam, a non-steroidal anti-inflammatory drug, during the recovery phase to complement the analgesic effects of buprenorphine. We reviewed the literature to determine known effects these drugs have on cats that were applicable to the work in this thesis.

Buprenorphine, a semisynthetic partial mu-opioid receptor agonist drug derived from oripavine and closely related to diprenorphine (M5050), was introduced into clinical practice in the 1970s (Cowan et al. 1977). When administered to cats, it did not alter the cats' behaviour (0.5 to 10 mg kg^{-1} subcutaneously). In mechanically ventilated halothane-chloralose anaesthetised cats, buprenorphine did not cause significant haemodynamic changes when administered at 0.1 to 1.0 mg kg^{-1} intravenously; it caused a non-significant decrease in heart rate when the higher doses were administered (Cowan et al. 1977). However, these high doses, which were initially administered to cats, are outside the clinically relevant range nowadays. Doses ranging from 0.02 to 0.04 mg kg^{-1}, preferably administered intramuscularly or intravenously, are considered clinically relevant analgesic doses in cats (Ilkiw et al. 2002; Robertson et al. 2005; Steagall et al. 2014). Oral transmucosal administration is also a relevant

route of administration while the subcutaneous route of administration is the least reliable and not advocated for clinical practice (Robertson et al. 2005; Steagall et al. 2014). Buprenorphine does not have an effect on the HPA axis (Gomez-Flores & Weber 2000; Franchi et al. 2007), has a minimal impact on cortisol secretion (Gomez-Flores & Weber 2000; Martucci et al. 2004) and no effect on natural killer cell activity or immune cell proliferation or cytokine activity (Gomez-Flores & Weber 2000; Martucci et al. 2004) in rats and mice, but the effect is assumed to be the same in cats. The effects of buprenorphine on the minimum alveolar concentration (MAC, described below) of isoflurane were statistically significant but not clinically relevant because the reduction was within the statistical error of the measured MAC (Ilkiw et al. 2002). In cats undergoing ovariohysterectomy, premedicated with buprenorphine (0.02 mg kg^{-1} intramuscularly) and maintained under general anaesthesia with isoflurane in oxygen following propofol induction, an increase in the end-tidal isoflurane concentration was required to maintain surgical anaesthesia when the ovarian ligaments were stretched (Bellini et al. 2017). The heart rate, respiratory rate and blood pressure increased and the $P_E'CO_2$ decreased when the two ovarian ligaments were stretched, which indicated that the sympathetic response to surgical stimulation was not supressed with this drug combination (Bellini et al. 2017). We concluded that buprenorphine (0.02 mg kg^{-1}) has minimal cardiovascular and respiratory effects, and isoflurane sparing effects, but maintains the sympathetic response to surgery. Also, buprenorphine is commonly used in cats and is recommended to treat acute pain and as a pre-anaesthetic drug in clinical practice (Steagall et al. 2014).

Alfaxalone is a synthetic neuroactive steroid anaesthetic drug that was first introduced into the market in the 1970s (Warne et al. 2015). The first formulation required the addition of a solubilising agent, called Cremophor-EL (polyethoxylated castor oil), which was known to stimulate mast cell degranulation and histamine release in animals (Whittem et al. 2008; Warne et al. 2015). Alfaxalone has a wide margin of safety, and therefore, research continued to identify a solubilising agent which could complement alfaxalone's safety margin. Nowadays, alfaxalone is formulated with a beta-cyclodextrin and is water-soluble, without any undesirable mast cell degranulation (Whittem et al. 2008). When administered alone at 5 mg kg^{-1} intravenously, cats calmly transitioned from an awake state to general anaesthesia and remained unresponsive to noxious stimulation (clamping of metacarpus or metatarsus for 10 seconds and base of tail for 60 seconds, or until a withdrawal response, using an intestinal clamp) for a mean (± SD) 7.2 ± 2.7 minutes, but took 45 minutes to lift their heads if left

unstimulated (Whittem et al. 2008). There were small decreases in the heart and respiratory rates; also, no apnoea was detected after administering the alfaxalone (Whittem et al. 2008). There was no evidence of pharmacokinetic or pharmacodynamic accumulation of alfaxalone, even on repeated doses (Whittem et al. 2008). The mean half-life of alfaxalone, when administering at this dose, ranges from 29 to 45 minutes (Whittem et al. 2008; Rodrigo-Mocholi et al. 2018). In healthy cats, dose-dependent decreases in heart rate, cardiac output, MAP and systemic vascular resistance have been described, but are short-lived and have minimal clinical relevance. There are also dose-dependent decreases in respiratory rate and minute volume, but they are also clinically irrelevant in healthy cats (Martinez Taboada & Murison 2010; Warne et al. 2015). These dose-dependent decreases have been reported in cats that were not administered a pre-anaesthetic drug, and that seemed anxious (heart rates >220 beats per minute; respiratory rates >50 breaths per minute); once anaesthetised their heart rates and respiratory rates decreased to a normal physiological range for cats (Whittem et al. 2008). Furthermore, the cardiovascular and respiratory effects of an intravenous bolus of alfaxalone (5 mg kg^{-1}) were minimal in buprenorphine (0.02 mg kg^{-1} intramuscularly; 20-30 minutes before alfaxalone induction) premedicated healthy cats (Bosing et al. 2012). During anaesthesia, the haematocrit, base excess and albumin concentrations decreased during alfaxalone-induced general anaesthesia, but the decreases were not clinically relevant (Bosing et al. 2012). In contrast to Bosing et al. (2012), Whittem et al. (2008) and Warne et al. (2015) reported that alfaxalone does not alter haematology of biochemistry analyte concentrations. Warne et al. (2015) also stated that the effects of alfaxalone on endocrine function and HPA-axis function remain to be investigated in cats.

Isoflurane is a volatile anaesthetic drug that belongs to the halogenated ether group of inhalation anaesthetics. It was introduced into clinical practice in the late 1970s and early 1980s. Isoflurane is a commonly used inhalation anaesthesia drug and there has been extensive research on its effects in cats. The minimum alveolar concentration (MAC) was defined and standardised by Quasha et al. (1980) in order to allow meaningful comparative investigations to take place, or to compare what another drug's effect is on the required MAC of inhalation anaesthetic drug. The classic definition of MAC is the minimum anaesthetic concentration, at sea level (1 atmosphere; 760 mmHg) required to prevent purposeful movement in 50% of subjects exposed to a standardised maximal noxious stimulation (Quasha et al. 1980). The major challenge in interpreting studies that have investigated MAC for various inhalation anaesthetic drugs among the species is that each study defines their noxious stimulation

(mechanical, thermal, chemical) and their endpoint (movement or no movement). Also, complicating the translation of knowledge from the bench to practice, is that age, sex, health status, pregnancy and body temperature are some of the variables that can alter the MAC and thus results of the study. Shaughnessy & Hofmeister (2014) reviewed the MAC for isoflurane in cats and determined it to be a mean ± SD of 1.71 ± 0.07%. In cats that were premedicated with ketamine and atropine and maintained under isoflurane (end-tidal isoflurane concentration 1.90 ±0.17%) anaesthesia had stable heart (150 beats minutes^{-1}) and respiratory (20 breaths minute^{-1}) rates (Hikasa et al. 1996). Additionally, the SAP, DAP and MAP were 100, 60 and 74 mmHg, respectively and stable (Hikasa et al. 1996). However, the packed cell volume and plasma proteins decreases compared to baseline readings (Hikasa et al. 1996). In a subsequent study, where no premedication was administered, cats were anaesthetised using isoflurane to achieve a MAC of 1.61% and then the cardiopulmonary effects of increasing MAC by 1.5 and 2.0 multipliers was investigated (Hikasa et al. 1997). In spontaneously breathing cats, as MAC was increased by the multiplier factors, the heart rate decreased from 160 to 147 to 135 beats per minute for MAC 1.0, MAC 1.5 and MAC 2.0, respectively. Similarly, the MAP decreased from 88 to 66 to 53 mmHg for MAC 1.0, MAC 1.5 and MAC 2.0, respectively. The respiratory rate decreased from 35 to 29 and 27 breaths per minute for MAC 1.0, MAC 1.5 and MAC 2.0, respectively (Hikasa et al. 1997). Similarly, Lerche et al. (2002) found that the heart and respiratory rate decrease during the induction of cats with isoflurane delivered by a face mask. Benson et al. (1991) found that isoflurane alone did not decrease the release of noradrenaline and adrenaline in cats undergoing onychectomy, which means that the sympathetic response to surgical stimulation remains intact. In dogs, where general anaesthesia was induced using alfaxalone and maintained with isoflurane (MAC for dogs, 1.4%), demonstrated that their cardiovascular and respiratory systems responded to severe haemorrhage and fluid resuscitation (McBride et al. 2017). Cardiovascular and respiratory responses were also maintained in isoflurane (end-tidal isoflurane concentration was between 0.7 and 1.1%) and fentanyl anaesthetised dogs undergoing severe haemorrhage and resuscitation (Driessen et al. 2006).

1.2.6 Fluids used to resuscitate absolute hypovolaemia

There have been many different fluids used to resuscitate the intravascular compartment after haemorrhage. They are broadly classified into crystalloid type and colloid type fluids (Rudloff & Kirby 1994).

A crystalloid fluid is defined as an aqueous solution of mineral salts, alone or with other water-soluble molecules of small molecular weight, like lactate ions that readily diffuse across plasma membranes. These solutions are formulated as hypotonic, isotonic or hypertonic relative to human plasma tonicity. The isotonic fluids are also formulated as unbalanced (0.9% physiological saline) or balanced (lactated Ringer's solution) solutions where the concentrations of the mineral salts in the solution are different or similar to normal human plasma concentrations of mineral salts, respectively (Rudloff & Kirby 1994; Rozanski & Rondeau 2002). Hypotonic solutions (0.45% saline or half-strength lactated Ringer's solution) are used for fluid maintenance therapy and are therefore not relevant to our study (Mazzaferro & Powell 2013). Hypertonic solutions (7.5% saline) have been used during various low-volume resuscitation protocols and have merit in fluid therapy, however, these solutions are not readily available in most general private practices and are usually used in academic or referral hospitals under the guidance of various specialist veterinarians. Therefore hypertonic solutions were not considered as treatments in our study. We selected the most commonly used isotonic fluid in South African veterinary practice, namely lactated Ringer's solution.

A colloid fluid is defined as a suspension of a high molecular weight oncotically (provides oncotic pressure, or colloidal osmotic force) active molecules suspended in an isotonic fluid. The high molecular weight molecules could either be synthetically derived (hydroxyethyl starch, gelatine or dextran based molecules) or be from natural compounds (albumin alone or various blood products). These large molecules could be suspended in concentrations that are higher than, or similar to, normal human blood oncotically active molecule concentrations and are called hyperoncotic or isooncotic fluids, respectively (Glover et al. 2014). Because of fairly recent government regulations on an international scale, many of the synthetic colloid solutions were withdrawn from the market (Jabaley & Dudaryk 2014). Currently, in South Africa, gelatin and hydroxyethyl starch-based fluids remain in use. Banking of cat blood products, like fresh whole blood or packed red blood cells, is not routine practice in South Africa and

these products are not readily available. Therefore, we selected a hydroxyethyl starch product (tetrastarch 6% 130/0.4; Voluven), which is the most commonly used synthetic colloid in South African veterinary practice as the colloid type fluid to investigate. The selected crystalloid and synthetic colloid fluids for our study are also readily available overseas and have been routinely recommended for use in cats (Rudloff & Kirby 1994; Rozanski & Rondeau 2002; Glover et al. 2014).

Whether to use crystalloids or colloids for resuscitation has been a topic of debate for many decades within the human medical practice (Jabaley & Dudaryk 2014), and to a less extent within the veterinary practice (Glover et al. 2014). This debate reached a pinnacle in 2013, when landmark studies and trials demonstrated that the administration of colloids, compared to crystalloids, increased the risk of death and the need for renal replacement therapy in septic or trauma human patients (Brunkhorst et al. 2008: VISEP study; Myburgh et al. 2012: CHEST study; Perner et al. 2012: 6S trial). These studies led to a short-lived international hype that reshaped the colloid products that are available today. Products were either withdrawn from the market entirely, or the package insert sheets were rewritten to include additional warnings of their use (Jabaley & Dudaryk 2014). The Cochrane Review examined the role of colloids for resuscitation in trauma, burn and surgical patients and concluded that: "*as colloids are not associated with an improvement in survival and are considerably more expensive than crystalloids, it is hard to see how their continued use in clinical practice can be justified*" (Perel et al. 2013). Review panels to identify and monitor pharmacovigilance risk assessments emerged in 2013, and their focus was to assess colloid (specifically the hydroxyethyl starches) use in humans and provide current guidelines which were instituted in 2018 (Adamik & Yozavo 2019). Despite concerns of administering synthetic colloid fluids raised almost a decade ago, they remain available for use today. Furthermore, the 2018 pharmacovigilance risk assessment committee (PRAC) review states that hydroxyethyl starches will not be removed from the market (Adamik & Yozavo 2019). The ability to translate veterinary study outcomes to human medicine using animal models has fallen out of favour (Jabaley & Dudaryk 2014). However, investigating colloid and crystalloid fluid therapy in dogs is ongoing, and the body of literature continues to grow (Boller & Boller 2015; Cavanagh et al. 2016; Thomovsky et al. 2016; McBride et al. 2017; Drozdzynska et al. 2018; Smart et al. 2018; Yam et al. 2019). In cats, however, there is a hiatus on fluid-related investigations, especially after severe haemorrhage. We speculate that each species handles haemorrhage and fluid resuscitation differently and information gained from one species may not be relevant in another species. Therefore, species-specific investigations should be

conducted to obtain meaningful findings that can be translated from bench to clinical practice. Furthermore, even in human medicine, it is not clear that conclusive evidence exist to prefer an isotonic crystalloid over a synthetic colloid for hypovolaemic resuscitation (Akech et al. 2010; Perel et al. 2013; Silverstein et al. 2012).

1.2.5.1 Lactated Ringer's solution

Sydney Ringer, a British physician and physiologist, invented the Ringer's saline solution in the early 1880s while studying extracorporeal frog hearts. The use of this balanced isotonic crystalloid solution gained popularity in use. In the early 1930s, an American paediatrician, Alexis Hartmann, added lactate to Ringer's saline to treat acidosis (Lee 1981). From then onwards the solution became known as lactated Ringer's solution and has remained unchanged since then; the solution also became known as Hartmann's solution (Wilkes 2003). Each manufacturer of this fluid produces a solution with a slight variation in its composition and, therefore, there can be small differences in its formulation. The differences are so insignificant that they are not considered relevant in clinical practice. For this study, we made use of lactated Ringer's solution manufactured by Fresenius Kabi. The formulation we used had the following composition and properties:

Sodium ion	131 mmol L^{-1}
Potassium ion	5.4 mmol L^{-1}
Calcium ion	1.8 mmol L^{-1}
Chloride ion	112 mmol L^{-1}
Lactate ion	29 mmol L^{-1}
Osmolality	278 mOsm L^{-1}
pH	6.0
Oncotic pressure	0 mmHg

1.2.5.2 Hydroxyethyl tetrastarch 130/0.4 fluid (Voluven)

The hydroxyethyl starch colloid fluids are made up of heavy, polydisperse, colloidally-active molecules derived from various starch polymers (amylopectin). There are varying numbers of hydroxyethyl residues that attach to an anhydrous glucose particle (Glover et al. 2014). The more hydroxyethyl residues attached to the glucose molecule, the more water-soluble it becomes; and the rate of destruction by amylase is decreased. The degree of substitution classifies the hydroxyethyl starch into its respective group (Westphal et al. 2009). The first product was Hespan and was made available in the 1970s. Hespan had a molar substitution of 0.7 which classified it as belonging to the hetastarch group and this made up the first generation of hydroxyethyl starches. Another factor for classifying these fluids was the average molecular weight of the suspended molecules, and it was thought that the higher the molecular weight the more effective the fluid was at restoring volume within the intravascular compartment (Westphal et al. 2009). The first generation molecules had an average weight of around 600 to 680 kDa. When the second generation of hydroxyethyl starches entered the market, there was a strong emphasis on classifying the colloid fluid, and thus each fluid has its average molecular weight followed by its substitution number; for example, Hespan 600/0.7. Another factor is the concentration of the suspended colloid within the isotonic solution (balanced or unbalanced); 6% indicates an isooncotic solution and 10% indicates a hyperoncotic solution (Westphal et al. 2009). The balance of molecular weight and molecular substitution varied for many decades in an attempt to find the ideal volume-expanding properties that would last longer than the crystalloids without causing untoward effects. However, the first two generations of hydroxyethyl starch solutions were prone to causing renal injury and coagulopathies, which was the beginning of the debate ‘colloids versus crystalloids’. The third generation of hydroxyethyl starches have low molecular weight (130 kDa) and low molecular substitution (0.4 and 0.42) and are classified as the tetrastarches (Glover et al. 2014). During their development and between the advent from the second to the third generation, another criterion of molecular structure became significant, the C2/C6 substitution ratio (Westphal et al. 2009). Amalyse cannot access the amylopectin substrate to destroy the molecule if there is a hydroxyethyl substitution at the C2 carbon of the glucose molecule, therefore instead of having a maximum substitution as originally thought, focus was placed on building a molecule that has the highest possible C2 substitution compared to the C6 molecule. The tetrastarches have a C2/C6 ratio of 9:1 and far exceed the usual

average 5:1 ratio of the first and second generation of fluids (Glover et al. 2014). Therefore, the tetrastarches are unique and do not have the same concerns and issues as seen with the previous generations of hydroxyethyl starches. They are less prone to causing renal injury and less likely to causing coagulopathies and, with this improved safety profile, they remain available for clinical use. Unfortunately, the tetrastarches are often condemned because they belong to the hydroxyethyl starch group of colloid fluids and have inherited their predecessor fluid safety profiles and concerns. Regardless of human medicine concerns, in dog and cat practice, there is no evidence that the tetrastarches cause acute renal injury or coagulopathies if administered at appropriate dose rates and used as volume expanders during hypovolaemia (Glover et al. 2014).

The available tetrastarch products are derived from different starch sources, potatoes or waxy maize. The potato-derived tetrastarches have a substitution of 0.42, whereas the waxy maize is 0.4, but both have an average molecular weight of 130 kDa (Bagchi & Eikermann 2013). There is a concern that the tetrastarches still cause acute renal injury and a range of possible explanations exist. The potato-based tetrastarch is high in phosphate (Bagchi & Eikermann 2013), which could, theoretically, cause a hyperphosphatemia which could be construed as an acute kidney injury. Additionally, if large volumes of these fluids are administered, they have been found to become trapped in the renal interstitial tissue. They could cause an interstitial nephritis, culminating in acute kidney injury. However, this effect has not been consistently reported in dogs (Diniz et al. 2018) and cats (Yozova et al. 2017); with the exception of a single case report in a dog that received several days of 6% tetrastarch 130/0.4 and manifested histological pathology of acute kidney injury that support nephrotoxic effects of hydroxyethyl starches (Bae et al. 2017). The first and second generation hydroxyethyl starches were known to cause issues with the glomerulus more often than the interstitial tissue of the kidney (Westphal et al. 2009). However, reports of acute kidney injury were in a subset of patients that received these fluids as maintenance fluids, in large volumes over days; these fluids are designed to be volume expanders to treat hypovolaemia and once the intravascular volume is restored their administration should be stopped. Also, the studies where renal replacement therapy was required in the patients that received a hydroxyethyl starch underwent major scrutiny over the last five years, and the aetiology of the acute kidney injuries that required renal replacement therapy were attributed to other causes and not directly linked to the hydroxyethyl starch fluid therapy (Westphal et al. 2009). Other, minor concerns have been

reported whereby some patients develop pruritus when tetrastarches are administered. However, this development has not been described in dogs and cats.

For the purposes of our study, we used the most common tetrastarch available to the South African veterinary market, which is called Voluven 6% (130/0.4) and has the following composition and properties:

Sodium ion	154 mmol L^{-1}
Chloride ion	154 mmol L^{-1}
Osmolality	308 mOsm L^{-1}
pH	4-5.5
Poly (0-2 hydroxethyl) starch	60.0 g L^{-1}
Molar substitution	0.38 – 0.45
Average molecular weight	130 kDa
Oncotic pressure	36 mmHg

1.2.5.3 Body response to fluid administration

With a century of information regarding fluid administration, there exists an overwhelming amount of information about what these fluids do to the body. There is a plethora of information on: 1) accumulation of fluids in body compartments and tissue storage; 2) effects on renal function; 3) effects on chemoreceptor function; 4) cardiovascular and respiratory effects and oxygen delivery; 5) myocardial perfusion; 6) effects on microcirculation and oxygenation; 7) effects on systemic inflammation and endothelial activation; 8) effects on haemostasis; and 9) effects on acid-base balance (Glover et al. 2014; Boller & Boller 2015; Cavanagh et al. 2016; Thomovsky et al. 2016; McBride et al. 2017; Drozdzynska et al. 2018; Smart et al. 2018; Adamik & Yozavo 2019; Yam et al. 2019). However, this list of topics is not exhaustive and a review of each of these topics is outside the scope of this thesis. What is lacking in the literature with regards to administering these fluids to cats that require volume resuscitation after severe haemorrhage is: 1) what are appropriate volumes to administer; 2) what are the cardiovascular, respiratory and oxygen content effects during fluids administration after severe haemorrhage; 3) what are the effects on haematology and renal and liver biochemistry; 4) what are the effects on acid-base balance; and 5) what are the effects of haemostasis.

1.2.7 Fluid administration protocols after severe controlled haemorrhage

Haemorrhage can be uncontrolled (continuously bleeding) or controlled (haemostasis achieved). Trauma patients usually present with uncontrolled haemorrhage due to slow insidious ongoing bleeding that is difficult to control medically or surgically (Niles 1999). Controlled haemorrhage is more typical of patients undergoing surgery, where, for whatever reason, the surgeon cannot maintain total haemostasis, resulting in a rapid (arterial bleeding) or slow (insidious seeping at site not related to the primary surgical site) bleeding event before gaining control of the haemorrhage (Niles 1999). Fluid administration to restore the intravascular compartment in trauma has evolved from initially administering 'shock doses' rapidly to achieve volume expansion and normotension, to a conservative approach where the patient is kept hypotensive until haemorrhage is controlled by giving smaller volumes of fluids over a longer period of time; this protocol is termed hypotensive resuscitation, or haemostatic resuscitation (Dutton 2012; Dunser et al. 2013). Once haemostasis is achieved, then the restoration of the intravascular volume is completed more rapidly with the aim of attaining normotension (Rudloff & Kirby 1994). This approach to fluid administration in uncontrolled haemorrhage is logical because rapid administration of fluids before haemostasis is achieved results in great intravascular fluid loss and impaired coagulation (Dutton 2012). However, during surgery, often the haemorrhage can be controlled and therefore more traditional resuscitation protocols can be used to restore the intravascular volume, and thus are relevant to this thesis (Rudloff & Kirby 1994; Rudloff & Kirby 2001; Rozanski & Rondeau 2002; Mapstone et al. 2003; Driessen & Brainard 2006; Mensack 2008; Glover et al. 2014). However, protocols to maintain fluid balance or correct electrolyte disturbances are outside the scope of this thesis. The biomarkers used to assess the response to fluid administration will be mentioned in this section but are detailed in '1.2.7 Biomarkers of adequate fluid resuscitation in companion animals' section next.

Many of these recent and clinically relevant resuscitation protocols have been shaped or derived from human emergency medicine and have been tailored for dogs and cats. However, most of the protocols have been investigated in dogs under research and clinical conditions; there is much less scientific evidence available for cats. Protocols for cats are often based on anecdotal evidence gained from decades of collective clinical experience and stem from the premise that cats are a fluid-sensitive

species and require special precautions during fluid administration. Furthermore, veterinary anaesthesia and emergency and critical care textbooks tend to recycle protocols and information that was known decades ago without much progression (Muir et al. 2017). Hypovolaemia is common during the intra-operative period, and goal-directed protocols have improved post-operative outcomes by decreasing morbidity in human medicine; this may be translatable to veterinary medicine (Gan et al. 2002). Fluid administration protocols and guidelines provide guidance to avoid known complications of fluid administration, such as fluid overload and haemodilution (Davis et al. 2013). In general, the fluid management of the animal would involve two phases, 1) the resuscitation phase where the intravascular compartment is replaced, and 2) the maintenance phase where fluids are administered to maintain the intravascular volume by replacing ongoing fluid losses. The various protocols discussed below are specific to the first phase only, the resuscitation phase (Rudloff & Kirby 2008). The purpose of this phase is to restore the adequate volume of the circulatory system, to prevent or treat hypovolaemic shock and support cellular metabolism (Rudloff & Kirby 1994)

1.2.6.1 The ‘shock dose’ protocol

Administering fluids at a ‘shock dose’ is arguably the simplest protocol to resuscitating the intravascular compartment in an emergency when there is hypotension associated with an absolute or relative hypovolaemia. The ‘shock dose’ is considered to be the administration of an isotonic crystalloid at one normal circulating blood volume per hour; in cats, that means a dose of 40-60 ml kg^{-1} per hour that is infused continuously (Rudloff & Kirby 1994; Rozanski & Rondeau 2002; Mazzaferro & Powell 2013). The volume expansion and retention within the intravascular compartment for isotonic crystalloids are less effective than for synthetic colloids, and therefore these fluids are not administered at ‘shock dose’ rates. Taking the volume expansion and intravascular retention of synthetic colloids into consideration, they have been recommended to be administered by infusion at 10-15 ml kg^{-1} per hour (Rozanski & Rondeau 2002). However, these doses are mostly based on first and second generation hydroxyethyl starches and the third generation tetrastarches have been inevitably been included within this dose range without investigation. Administering isotonic fluids at this dose has resulted in post-resuscitation complications like fluid overload and haemodilution. Because of these complications, the ‘shock dose’

fluid administration protocol has become the quintessential example of a liberal fluid resuscitation protocol. Driessen and Brainard (2006) stated that information on the use of synthetic colloids in small animals is sparsely available more than a decade ago, and that currently a maximum of 10 ml kg^{-1} per day should be administered to cats. This lack of evidence and lack of translation into clinical practice, even with crystalloid fluid therapy, remains a concern in veterinary medicine (Muir et al. 2017). In humans, tetrastarches have been administered up to 50 ml kg^{-1} per day, and up to 70 ml kg^{-1} per day in research settings without adverse effects (Gandhi et al. 2007; Cada et al. 2008). In cats, synthetic colloids could be administered at 10-40 ml kg^{-1} per day (Mensack 2008). The 'shock dose' protocol should only be used during the immediate-early phase of volume resuscitation, and once life-threatening hypovolaemic-hypotension is resolved, then the fluid should be administered using a more conservative protocol (Hammond & Holm 2009).

1.2.6.2 The crystalloid 3:1 and colloid 1:1 protocols for treating haemorrhage

It has been recommended to administer crystalloids and synthetic collides at a 3:1 and 1:1 ratio of the volume of blood loss, respectively. In other words, for every 1 ml of blood lost, the animal should be administered 3 ml of isotonic crystalloid or 1 ml of synthetic colloid to replace the volume lost within the intravascular compartment.

The origin of the 3:1 ratio rule for isotonic crystalloid administration stems from controlled haemorrhage animal research (dogs, pigs, goats and sheep) in the 1950s and 1960s. These investigators observed that the animals were more likely to survive if isotonic crystalloids were infused at 2-3 times the ongoing blood volume loss during prolonged haemorrhage-induced hypotensive experiments (Broadstone 1999). Despite the 3:1 ratio rule still being advocated nowadays, its application in clinical practice has been questioned (Lijima 2009). In clinical practice, more than a 3:1 ratio (often 4:1 or 5:1) is required to obtain the same volume expansion to replace that of the blood lost; this large volume inevitably leads to post-resuscitation complications (Rehm et al. 2019). A recent rat-based model research suggests that a 1:1 ratio should be used for isotonic crystalloid resuscitation, having found that the rats had adequate compensatory capacity and no pulmonary oedema (Fodor et

al. 2019). If isotonic crystalloids are administered immediately after haemorrhage, than only a 1.6:1 ratio is required to restore the intravascular volume (Hahn 2013).

The origin of the 1:1 rule for synthetic colloid administration stems from volume expansion of a isooncotic synthetic colloid which is approximately 100% of the volume of blood lost (Broadstone 1999; Rehm et al. 2019). Because synthetic colloids are more effective and quicker at restoring the intravascular volume compared to isotonic crystalloids, they have been recommended as the resuscitation fluid of choice when more than 20% of the circulating blood volume is lost (Broadstone 1999). This recommendation stems from the fact that colloids have a longer dwell time within the intravascular compartment compared to crystalloids and are therefore more effective for volume resuscitation. The increased dwell time is because the starch molecules are negatively charged and attract positive ions, and ions like sodium attract water. This effect is similar to a Gibbs-Donnan effect (Nguyen & Kurtz 2006). Despite the ongoing 'crystalloids versus colloids' debate, many agree that synthetic colloids do have a clinically relevant place during immediate resuscitation of the intravascular compartment due to severe relative or absolute hypovolaemia.

The most important problem with the ratio rules is that the volume of blood being lost needs to be known, so that the appropriate amount of fluid can be administered, according to the ratio. In surgical patients, the volume of blood being lost is often monitored subjectively or measured exactly; in this scenario, the ratio rules could be applied. The second problem is that the actual volume (and therefore ratio) of fluid required differs depending on how quickly the fluid is administered after controlled haemorrhage (Hahn 2013). Generally, if fluids are administered immediately, then less fluid is required to achieve a normovolaemic state. However, the rate of fluid administration also plays a significant role; the original 3:1 ratio rule was established in haemorrhage-induced hypotensive research models that lasted more than 4 hours. Therefore, the dwell time of the crystalloid or synthetic colloid fluid within the intravascular compartment becomes an important criteria to consider. Crystalloids will move out of the intravascular compartment more readily compared to synthetic colloids, but the rate of movement is dependent on how volume-depleted the intravascular compartment is. These problems and considerations complicate the development of an ideal fluid administration protocol.

These two traditional liberal fluid administration protocols have been brought into question decades ago, and in the early 1980s other protocols began to emerge (Hammond & Holm 2009). Furthermore,

specifically in cats, the risk of volume overload, pleural and pulmonary effusions and haemodilution are real. Thus modifications to these fluid administration protocols have been advocated for routine practice (Davis et al. 2013). The modifications to the protocols could be termed restrictive, conservative, limited volume, small volume or endpoint resuscitation protocols (Glover et al. 2014).

1.2.6.3 Modified traditional protocols

The risk of inadvertently causing fluid overload and the sequelae of tissue oedema and cavity effusions is high when the traditional protocols are used. Therefore, adjustments to the traditional protocols; or even hybrid fluid protocols have been used to try to prevent or minimise these sequelae.

The 'shock dose' protocol has been revisited, and the modified approach has been very well accepted, is simple to implement and comes highly recommended to use (Davis et al. 2013). This modified approach is where the animal is administered smaller doses as boluses over 5-10 minutes and separated by a waiting period of 10-15 minutes. Thus titrating the administered fluid volume according to a resuscitation end-point biomarker. Typically, one-quarter to one-third of the 'shock dose' is administered per bolus. For cats a bolus of 15-20 ml kg^{-1} of an isotonic crystalloid can be given at a time (Driessen & Brainard 2006; Rudloff & Kirby 2008). Minor variations on the dose-volume and the time required to administer the dose exist, but the overall sentiment of the protocol remains the same. The bolus protocol modification allows the veterinarian a more hands-on approach, which means there is better monitoring of the effect that the fluid has on the cat compared to initiating an infusion at the shock dose. However, the total shock volume of fluid could still be given, and therefore it is not much different to just infusing the dose and observing the response to fluids. Furthermore, Krausz et al. (2003) reported that, in rats after severe splenic injury, a splenectomy to control further blood loss and a constant rate infusion of lactated Ringer's solution improved survival and haemodynamic stability compared to the group of rats that received intermittent boluses of the same fluid. For synthetic colloids, small boluses of 2.5 to 3 ml kg^{-1} should be administered over 10 minutes and then titrated upwards to clinical effect (Glover et al. 2014). It has been commonly suggested not to exceed a total dose of 10-15 ml kg^{-1} in cats (Davis et al. 2013); this recommendation was based on first and second generation hydroxyethyl starches and it is unknown if this is relevant for the tetrastarches. Synthetic colloids are

the most effective at resuscitating the hypovolaemic intravascular compartment compared to isotonic crystalloids (Davis et al. 2013). Regardless of which fluid is administered to a cat, a very important consideration is the body temperature; cats should be warmed to a normal body temperature while slowly administering the fluids to prevent accidental fluid overload and pulmonary oedema (Driessen & Brainard 2006; Rudloff & Kirby 2008; Cavanagh et al. 2016; Ostroski et al. 2017).

The hybrid fluid protocols are when two or more different types are fluids are administered to replace the lost intravascular volume. In cases of simple haemorrhage-induced hypovolaemia, often a single fluid type is advocated to restore the intravascular volume. However, more complex trauma or critically ill cases may require a more complex fluid management plan that invariably results in the administration of more than one fluid type to achieve resuscitation goals. In cats, it has been advocated to administer a synthetic colloid and isotonic crystalloid combination at 1-5 ml kg^{-1} and 25-27 ml kg^{-1}, respectively (Davis et al. 2013). It is recommended to bolus these fluids until the desired clinical effect has been achieved. Furthermore, although outside the scope of this thesis, hypertonic crystalloids like hypertonic saline have been administered in combination with isotonic crystalloids, or synthetic colloids, or both (Driessen & Brainard 2006). Any fluid administration protocol using hypertonic saline often has terms like low volume, restricted volume or small volume describing the protocol; hypertonic saline is a very useful fluid to use in the initial phase of resuscitation from haemorrhagic shock.

These protocols, liberal or modified (restrictive), are easy to follow and are lifesaving to treat absolute and relative hypovolaemic hypotension and therefore are often recommended to be used in private practice situations. However, fluid management can be (and is) much more complex; for effective and safe fluid administration, the veterinarian will require advanced knowledge of blood, the fluid compartments and the different fluids to compile an individualised fluid protocol (Driessen & Brainard 2006). Regardless of the protocol used, it is critical to monitor the response to the administration of fluids and to titrate fluid administration doses to the desired clinical effect. There are many static and dynamic biomarkers that have been proposed to signal an endpoint for fluid resuscitation in veterinary science (Rudloff & Kirby 2008).

1.2.8 Biomarkers of adequate fluid resuscitation in companion animals

Biomarkers used to determine if fluid resuscitation has adequately restored the intravascular fluid volume can be divided into cardiovascular system-related and oxygenation related biomarkers (Marik et al. 2011; Dunser et al. 2013; Pinsky 2015). The biomarker endpoint targets of resuscitation include variables that reflect adequate body homeostasis by ensuring, at a minimum, adequate oxygen is delivered to the metabolising tissues. Biomarkers used to determine fluid responsiveness have also been described where the aim is to evaluate if there is a response to fluid therapy (Pinsky et al. 2008); however, these biomarkers are not well established in veterinary practice (Prittie 2006; Rudloff & Kirby 2008; Drozdzynska et al. 2018). The earliest reported biomarkers were cardiovascular system related. As the pathophysiology of absolute and relative hypovolaemia (hypovolaemia shock) became better understood, then the oxygenation related biomarkers became of interest. Many of these biomarkers have been used as 'goal-directed' endpoints of resuscitation regardless of the fluid administration protocol being used (Bloomstone et al. 2017). In animals, it is presumed that early resuscitation results in a decrease in the risk of death; and in humans, the strategy of administering fluids to achieve target biomarker endpoints is termed 'goal-directed fluid therapy' and was first suggested in the early 1980s (Shoemaker et al. 1983; Gan et al. 2002). Simple absolute hypovolaemia because of haemorrhage, in theory, should be easier to monitor for biomarker endpoints compared to patients already in shock or otherwise critically ill.

The traditional biomarkers used during resuscitation require keen clinical observation and equipment that is available to all veterinarians. These are variables that can be monitored during and after fluid administration to detect a response (change in variable value) and to indicate when to discontinue the resuscitation phase of fluid administration. These traditional variables include: 1) perfusion parameters (mentation, heart rate, peripheral pulse quality, capillary refill time, mucous membrane colour, rectal temperature) 2) systemic arterial blood pressure, and 3) urine output (Hammond & Holm 2009). However, in humans, even when these variables have returned to values within normal reference intervals, up to 85% of severely injured patients still have evidence of ongoing tissue hypoxia, occult oxygen debt and compensated shock (Prittie 2006). Some of the perfusion parameters are not helpful in animals that are under general anaesthesia, such as mentation. In the case of general anaesthesia,

the recovery time might be a more critical parameter to assess. Poor perfusion to organs of excretion such as the kidneys or the liver will impact drug metabolism and elimination negatively, prolonging the recovery time. Similarly, inadequate perfusion under general anaesthesia will interfere with warming effort to bring/keep the patient in its normal reference interval, influencing negatively on drug metabolism as well as time to return to consciousness. Heart rate, pulse quality, systemic arterial blood pressure and urine output are biomarkers that can be monitored while the animal is under general anaesthesia. However, drugs might affect these variables and render their usefulness questionable during peri-anaesthetic fluid resuscitation. For example, opioid receptor agonists can cause oliguria; isoflurane can cause vasodilation which decreases the systemic arterial blood pressure and increases the heart rate in an attempt to normalise the blood pressure; alpha2-adrenoceptor agonist drugs (medetomidine and dexmedetomidine are commonly used in cats) can increase the systemic arterial blood pressure and cause a baroreflex decrease in heart rate and cardiac output; ketamine (a commonly used cyclohexylamine dissociative anaesthetic drug) can cause in increase in systemic arterial blood pressure and heart rate though indirect stimulation of noradrenaline release. Also, as mentioned in the section '1.2.3 The physiology of haemorrhage in cats', cats can maintain their systemic arterial blood pressure above target values despite losing almost their entire circulating volume and being very close to irreversible cardiovascular collapse through compensatory intense vasoconstriction. Therefore, if systemic arterial blood pressure is used alone as a biomarker then cats could be under-resuscitated (Driessen & Kirby 2006; Prittie 2006; Rudloff & Kirby 2008). The usefulness of these traditional variables that are commonly used to guide fluid resuscitation might be very limited in cats. More sophisticated pressure monitoring techniques have been used in anaesthetised animals to determine their responsiveness to fluid resuscitation and include central venous pressure and advanced arterial waveform analysis during spontaneous or mechanical ventilation (Marik et al. 2009).

Central venous pressure monitoring can be done in anaesthetised animals and is a popular monitoring technique that some veterinarians still find useful today (Self 2015a; Hutchinson & Shaw 2016). The reference interval of central venous pressure in cats ranges from 0 to 10 cmH_2O (Self 2015a). The central venous pressure is an indicator for left ventricular end-diastolic volume, which is the preload. The resultant pressure is an indicator of how well the venous return is interacting with cardiac function. While a mean central venous pressure less than 1 cmH_2O might be seen as a sign of hypovolaemia a mean greater than 12 cmH_2O is more likely to correlate with fluid overloaded or

hypervolaemia (Hutchinson & Shaw 2016). If the cat is thought to be hypovolaemic then a fluid challenge has been advocated. To perform a fluid challenge, a bolus of fluid (10 ml kg^{-1}) is administered over 10 minutes and then the central venous pressure is measured (Self 2015a). If the cat is hypovolaemic than there will be a transient rise in central venous pressure by up to 3 cmH_2O that returns to the pre-challenge value within 5 minutes. If the cat normovolaemic, then the rise in central venous pressure will be greater (greater than 5 cmH_2O) and last longer than 15 minutes (Self 2015a). There are a number of limitations to its clinical application. One of which is measuring central venous pressure under normal conditions. Ideally, the patient should be placed in lateral recumbency and the measurement should be taken during the expiratory pause that occurs between breaths (Hutchinson & Shaw 2016). Thus, a respiratory rate of 8 breath per minute allows for enough time to obtain a measurement. However, conscious cats cannot easily be physically restrained in lateral recumbency, nor do they have a slow enough respiratory rate when restrained to obtain an accurate measurement. Therefore, this measurement is easier to perform in heavily sedated or anaesthetised or moribund cats. Despite central venous pressure still being advocated as a useful biomarker, there is evidence that it is a very late indicator of fluid overload (Marik et al. 2008). Central venous pressure, arterial blood pressure and heart rate are all insensitive biomarkers of hypovolaemia and fluid resuscitation and cannot be encouraged for meaningful clinical application (Marik et al. 2008; Duffy et al. 2009; Marik et al. 2009; Drozdzynska et al. 2018). Therefore, search in finding techniques that are more sensitive and reliable lead to the study of advanced arterial waveform analysis.

Advanced arterial waveform analysis includes systolic pressure variation and pulse pressure variation measurements (Marik et al. 2009; Duffy et al. 2009; Pinsky 2015; Drozdzynska et al. 2018). These are ideally used in anaesthetised (or moribund) and mechanically ventilated animals. To obtain a measurement, an artery must be cannulated and the arterial waveform should be displayed on a time graph. Some monitoring machines have internal algorithms to calculate the variable of interest, however, if this is not possible then a printed version of the waveform can be used and the variables calculated manually. For systolic pressure variation, the systolic pressure is determined during at least two respiratory breath cycles, and the difference between the highest recorded pressure and the lowest recorded pressure is calculated (Pinsky 2015; Araos et al. 2020). During mechanical ventilation, the systolic pressure will be at its highest during the inspiratory phase of the breath, whereas during spontaneous ventilation the systolic pressure will be at its highest during the expiratory phase of the

breath (Pinsky et al. 2008; Pinsky 2015; Araos et al. 2020). Furthermore, the oscillating variation around the average systolic pressure measured during a 5 second respiratory pause has been used as a zero point and the change in systolic pressure above this zero point is the delta up; the change in systolic pressure under the zero point is the delta down (Pinsky 2015). Therefore the calculated systolic pressure variation can be divided into two components, the delta up and the delta down. No reference intervals are currently published for dogs or cats, but in dogs the systolic pressure variation provides a good indication of volume status and can reliably identify dogs that are hypovolaemic (Drozdzynska et al. 2018). The pulse pressure variation technique requires the same waveform tracing over at least two breath cycles (Lamia et al. 2005). In this technique, the pulse pressure of the maximum and minimum pulse waves is calculated (Araos et al. 2020). Then the minimum pulse pressure is subtracted from the maximum pulse pressure to obtain the change in pulse pressure (delta pulse pressure). The pulse pressure variation is then converted to a percentage by dividing the delta pulse pressure by the mean pulse pressure. This technique has also shown promise in becoming a useful monitor of volume status in dogs. Another technique relies on contour analysis of the arterial waveform tracing and is used to calculate the stroke volume using complex proprietary algorithms (Pinsky 2015; Araos et al. 2020). Briefly, the area under the systolic phase of the arterial pulse wave graph (from the upstroke to the dicrotic notch) is calculated to determine the stroke volume. The maximum and minimum stroke volumes are calculated, and then the difference between them is calculated; this difference is then divided by the mean stroke volume to obtain a percentage value. This technique demonstrates reliable measurements and can identify hypovolemic dogs. However, this technique and the pulse pressure variation technique require the animal to be mechanically ventilated and there are no reference intervals (only inferences from human studies) available to guide therapy in dogs and cats (Duffy et al. 2009; Pinsky 2015; Drozdzynska et al. 2018; Araos et al. 2020). Despite these novel techniques showing promise in animals, the practicality of having to mechanically ventilate the animal precludes the overall usefulness in private practice, or in animals that do not require mechanical ventilation. All pressure measurements have the downfall of not directly measuring the flow of blood. In other words, you can have an adequate systemic arterial blood pressure, but still have a decreased cardiac output because of intense vasoconstriction, as per the arterial blood pressure equation.

In 1928, Adolf Jarisch stated that "*It was fatal for the development of our understanding of circulation that blood flow is relatively difficult while blood pressure so easy to measure: This is the reason why the*

sphygmomanometer has gained such a fascinating influence, although most organs do not need blood pressure but flow." (Jarisch 1928). There are flow variables that have also been used to monitor fluid responsiveness and resuscitation.

Cardiac output monitoring is commonplace in human critical care medicine and techniques range from non-invasive to very invasive (Pinsky et al. 2008). These human-based techniques have been used in dogs, mostly under experimental conditions, or in academic and large referral hospitals. Cats, because of their small body size, are prone to technical challenges whereby the catheters are often too large, which poses an increased risk of complications. Also, the less invasive techniques do not perform reliably in dogs, and it is speculated that this would be true for cats too. Therefore, cardiac output monitoring in cats is a challenge. A measure of cardiac output can indicate the global perfusion and blood flow status of the animal but not necessarily specific organ perfusion and blood flow (Pinsky et al. 2008). There are barriers to mainstream use of cardiac output in veterinary patients, and the most important consideration is the cost of using the reliable invasive techniques, such as placement of a pulmonary artery catheter. While this technique is still regarded as the gold standard, for human monitoring, often minimally invasive pulse contour analysis techniques are used (Pinsky et al. 2008). However, these techniques have not shown reliability in dogs and are therefore not considered for clinical application. The other concern with using the pulmonary artery catheter is that it requires an indicator (ice cold fluids or lithium) to be injected into the right atrium and a sensor on the catheter tip within the pulmonary artery to determine the lithium concentration or the change in temperature; these values are used to calculate the cardiac output using various algorithms based on the Stewart-Hamilton equation (Argueta & Paniagua 2019). For best results, the readings should be obtained in triplicate, and thus these techniques are static (recorded at set intervals according to the clinician's orders) and require the administration of additional fluids, which is not ideal for research on fluid resuscitation. Therefore, these techniques were not seen as useful for this thesis. A minimally invasive technique used to determine cardiac output is the Fick method (Argueta & Paniagua 2019). The Fick method has been described for cardiac output monitoring in cats and is still seen as the gold standard technique in that species (Baxter et al. 1952). However, it requires the patient to rebreathe their exhaled respiratory gases for 1 minute, which in our study design was not an easy additional variable to include. Modern minimally to non-invasive techniques have begun to emerge and they have been tested in dogs with success. One such technique is where the arterial plethysmograph of a pulse oximeter is plotted and

the area under the pulse wave is calculated using proprietary algorithms (Drozdzynska et al. 2018; Araos et al. 2020). The variation in the area under the graph indicated the intravascular volume status of the patient; if there is a lot of variation during spontaneous (or preferably mechanical) ventilation then the animal is said to be volume depleted. Whereas, if there is little variation during the respiratory cycle then the animal is normovolaemic. The concept of this technique is similar to that of the arterial pulse contour technique. However, in cats, because their tongues have thick hair-like projections and their peripheral arteries are small in diameter, often these plethysmograph plots are prone to error and overcorrection. Until this technique has been validated for experimental purposes to indirectly monitor cardiac output it could not be used in our study. Despite not being able to monitor blood flow or cardiac output in our study, we could monitor downstream variables that can provide an indication of adequacy of blood flow and these variables are used to monitor the oxygenation status of the body and are therefore the oxygenation biomarkers.

One of the oldest indicators of oxygenation status is the measurement of lactate (Prittie 2006; Rosenstein et al. 2018a). Although there are many mechanisms for lactate production, most of them are related to anaerobic metabolism. Lactate can be increased in states where there is inadequate oxygen delivery to the metabolising tissue or when there is a rapid increase in metabolism which quickly uses up oxygen or co-enzymes that are required in aerobic metabolism (Rosenstein et al. 2018a). The lactate pathway of energy production is essential and it is the most efficient pathway during states of anaerobic metabolism. If severe haemorrhage occurs then the haemoglobin concentration is expected to decrease which will result in a decrease in the oxygen carrying capacity of blood. Therefore, during haemorrhage-induced hypovolaemia, an oxygen debt could quickly develop, leading to haemorrhagic shock. Lactate has been a consistent substrate to measure and has been investigated as a prognostic indicator (Rosenstein et al. 2018b). The change in lactate from a high concentration to a value that is within the normal reference interval for the species (in dogs and cats, often considered as being less than 2 mmol L^{-1}) is a good indicator of adequate fluid resuscitation (Rosenstein et al. 2018b). Other indicators of oxygenation are the central and mixed venous oxygen saturations, where a sample is obtained from the intrathoracic cranial vena cava or the pulmonary artery catheter, respectively, and measured using a blood gas analyser to determine the oxygen saturation of haemoglobin (Walton & Hansen 2018). If the central venous and mixed venous oxygen saturation is less than 65% and 70%, respectively, then this indicates that too much oxygen is being extracted from the metabolising tissues

and that an oxygen debt likely exists (Prittie 2006). The mechanism that causes the increase in oxygen extraction during hypovolaemia-induced hypotension is because of the decrease in cardiac output.

There are many more biomarkers that have been investigated in human and veterinary medicine, but to date, none appear to be the 'gold standard', worthy and reliable in all cases and clinical presentations (Pinsky 2015). A complete mention of all possible biomarkers is outside the scope of this thesis. In the context of the biomarkers mentioned in this section are relevant and have been advocated in veterinary medicine, fluids are administered until the desired target is achieved and each individual reaches a biomarker target at different volumes of fluids used to resuscitate the intravascular volume. Unfortunately, these biomarkers do not easily predict fluid responsiveness in clinical practice compared to research scenarios, and thus the risk for causing inadvertent volume overload is always present. Selecting the best biomarker to use remains controversial, and there is not enough clinical evidence within veterinary practice to make strong recommendations for choosing one biomarker over another (Prittie 2006). Therefore, until such a biomarker exists, it is a common recommendation to utilise and assess a combination of biomarkers to guide resuscitation fluid therapy (Prittie 2006). However, current biomarker endpoints of resuscitation can (and often do) result in iatrogenic fluid overload (Cavanagh et al. 2016), thus volume endpoints need to be defined. Volume endpoints could indicate when enough fluid volume has been administered and that, if the patient has not reached an adequate resuscitation endpoint, then, perhaps, catecholamine drugs might be required to achieve these resuscitation endpoint goals instead of more fluids. To date, there are no real consensus-driven maximum volumes that should be administered and often the 'shock dose' has been used as a general indication of the maximum volume of fluid that should be delivered during the resuscitation phase. However, even with this guideline, fluid overload is still possible. The most challenging question that remains unanswered is "how much fluid do you administer before you decide the animal is a fluid non-responder?". Fluid non-responders have many co-morbidities responsible for the lack of response, a complete discussion is outside the scope of this thesis, and these animals are more likely to succumb to the sequelae of liberal fluid administration, such as pulmonary oedema, tissue oedema, dilutional coagulopathies, tissue hypoxia and general volume overload (Cavanagh et al. 2016).

1.2.9 Acid-base theory of blood

Acid-base theory has been an active field of medical research for more than a century and there are now many methods used to analyse blood acid-base balance. For the purposes of this thesis we will focus on the three most commonly published analysis methods used in veterinary medicine by anaesthesiologists and criticalists, namely: 1) the Henderson-Hasselbalch method, 2) the Stewart method, and 3) the semi-quantitative method (Hopper et al. 2014a).

The important events and milestones that have shaped blood acid-base theory began in 1908, when Lawrence Henderson described an equation that could be used to calculate the pH of a buffer solution (Henderson 1908). Then, in 1917, Karl Hasselbalch applied logarithmic functions to Henderson's equation (Hasselbalch 1917), resulting in the development of the Henderson-Hasselbalch method of pH analysis of solutions. This method has been the cornerstone of interpreting blood pH for a century. The concept of base excess (BE) was developed by Poul Astrup and Ole Siggard-Anderson and colleagues in 1960 (Astrup et al. 1960). In the 1970s, the advent of the anion gap (AG) was developed, which significantly improved the ability to diagnose and treat acid-base imbalances (Ohms 1977). Peter Stewart, in 1983, was the first to propose a modern quantitative method of describing acid-base status (Stewart 1983). Stewart's method was based on physical chemistry principles which included electroneutrality, conservation of mass and the dissociation of electrolytes (Russell et al. 1996; Hopper 2015a). He proposed that overall pH is controlled by three independent variables: PCO_2, the strong ion difference (SID), and total week acids (Atot). The hydrogen and bicarbonate ion (HCO_3^-) concentrations are dependent on these three independent variables (Hopper 2015b). The Stewart method was aimed to describe the mechanisms and quantify acid-base disorders more completely compared to the Henderson-Hasselbalch method. Furthermore, the Stewart method has been used to theoretically predict and describe changes in acid-base status during fluid resuscitation and thus improve our understanding of case management (Muir 2017). Vladimir Fencl and colleagues combined concepts from the Henderson-Hasselbalch and Stewart methods to derive the semi-quantitative method (Fencl et al. 2000). The semi-quantitative method makes use of quantifying the effects of individual acid-base processes on the base excess. These processes include the effects of albumin, chloride, phosphate, sodium and lactate concentrations (Fencl et al. 2000). This method, which examines many processes,

can detect acid-base disturbances in cases presenting with normal blood pH according to the Henderson-Hasselbalch and Stewart methods (Hopper et al. 2014a).

The Henderson-Hasselbalch approach relates the blood pH to the constituents of the bicarbonate buffering system ($CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$) using the following equation (Henderson 1908; Hasselbalch 1916):

$$pH = pKa \text{ of } H_2CO_3 + \log_{10} ([HCO_3^-]/[H_2CO_3])$$

Which has been adapted for clinical application by the following equation (Constable 2000):

$$pH = pK_1' + \log_{10} ([HCO_3^-]/S.PCO_2)$$

where pK_1' is the equilibrium dissociation constant of carbonic acid = 6.105 at 37.0°C (human); *S* is the solubility coefficient of carbon dioxide in plasma = 0.0307 [mmol L^{-1}]/mmHg.

The two organ systems that play an important part in balancing the bicarbonate buffer system of the blood are the pulmonary and renal systems (Hopper 2015a). The pulmonary system is responsible for gas exchange where carbon dioxide exits the capillary blood vessel into the alveoli and eventually is exhaled (measured in exhaled gases using capnography or dissolved in the plasma by blood gas analysis), and is responsible for the respiratory component to acid-base balance. The renal system can absorb and excrete bicarbonate ions, depending on the blood pH, and is responsible for the metabolic component to acid-base balance (Hopper 2015a). If the PCO_2 is less than or greater than the normal reference interval for a particular animal species then they are said to have a respiratory alkalosis or respiratory acidosis, respectively. Similarly, if the HCO_3^- concentration is less than or greater than the normal reference interval for the particular animal species then they are said to have a metabolic acidosis or metabolic alkalosis, respectively. The normal reference interval of the venous partial pressure of carbon dioxide ($PvCO_2$) for cats is 34 to 39 mmHg; and 18 to 26 mmol L^{-1} for HCO_3^- (Hopper et al. 2014a). Examining the clinical version of the pH equation, the $[HCO_3^-]$ is divided by the PCO_2 which forms a ratio of the metabolic to respiratory components; the body will attempt to maintain a ratio of 20:1 (Robertson 1989). If there is an increase in PCO_2 (respiratory acidosis) then there should be a reciprocated increase in HCO_3^- (metabolic alkalosis) in order to keep the ratio as close to 20:1. This ratio response is usual for simple acid-base disturbances and is the basis of the body's compensatory response to the primary acid-base disturbance (Hopper 2015a). However, mixed disturbances can also

occur whereby the PCO_2 and HCO_3^- move in opposite directions, and no compensation occurs. The respiratory component of acid-base balance occurs in minutes, therefore the change in ventilation can happen within a very short time period to adjust the PCO_2 level. The metabolic component of acid-base balance occurs in hours to days, therefore the change in renal absorption or excretion of HCO_3^- is slow and thus metabolic disturbances can take time to correct (Hopper 2015a).

The pulmonary and renal systems play a large role in acid-base balance but the participation of haemoglobin cannot easily be excluded. Haemoglobin can act as an oxygen transporter or as a buffer, and its buffering capacity is related to the bicarbonate buffer system. When the erythrocyte traverses the systemic capillary bed, there is a high PCO_2 which encourages the haemoglobin to offload oxygen and take up the CO_2 either as a buffer or CO_2 transporter, through the Bohr effect (Dugdale 2010). The CO_2 moves into the erythrocyte and binds with water to form carbonic acid and the acid quickly dissociates into a hydrogen ion and HCO_3^-. The hydrogen ion binds to haemoglobin to be buffered and HCO_3^- builds up within the cytosol of the erythrocyte. For the process to continue, the HCO_3^- needs to exit the erythrocyte cytosol to enter the plasma, and it does this by a chloride-HCO_3^- exchange to maintain electrical neutrality (Dugdale 2010). Chloride will move into the erythrocyte and HCO_3^- will move outward. This exchange of anions is termed the chloride shift or Hamburger phenomenon, named after Hartog Jakob Hamburger (Hamburger 1918). Therefore, when haemoglobin acts as a buffer, there is an immediate release of HCO_3^- into the plasma which could account for the rapid rise in plasma HCO_3^- despite the renal metabolic compensation taking hours to days to occur. This rapid rise in plasma during respiratory acidosis has been documented in chemically captured impala (Zeiler & Meyer 2017), and we speculate that this haemoglobin mechanism of rapid compensation has not been well explored in dogs and cats.

The Henderson-Hasselbalch method is descriptive in nature, whereby acid-base disturbances can be acidic or alkalotic and classified as being respiratory or metabolic in process. To improve the metabolic classification (acidotic or alkalotic) further, the BE and AG could also be considered (Hopper et al. 2014a). Together, these methods are considered the traditional method of acid-base balance analysis. The use of HCO_3^- or BE to interpret the metabolic component of an acid-base disturbance has been debated to such an extent that Bunker introduced the expression “the great transatlantic acid-base debate” (Bunker 1965). This debate is outside the scope of this thesis because we will consider analysing both HCO_3^- and BE in the cats; also, modern benchtop blood gas analysers calculate the

different values for HCO_3^- and BE to allow the practitioner to evaluate the acid-base balance according to their preference (**Table 1.2**). The important aspect of the different formulae used is that standard HCO_3^- and blood BE incorporate the haemoglobin concentration as an equation variable, and this variable is relevant in cats that undergo severe haemorrhage and resuscitation because the haemoglobin concentration is expected to decrease.

Table 1.2 Benchtop blood gas analyser machine (RapidPoint 500; Siemans; machine used in study) formulae used to calculate the actual [log HCO_3^-(act)] and standard [HCO_3^-(std)] bicarbonate ion concentration and extracellular fluid [BE(ecf)] and blood [BE(B)] base excess.

Parameter	Formula
HCO_3^-(act)	$pH + \log (PvCO_2 \times 0.0307) - 6.105$
HCO_3^-(std)	$24.5 + 0.9A + (A - 2.9)^2 \times (2.65 + 0.31 \times Hb)/1000$
	Where: $A = BE(B) - (0.2 \times Hb \times (100\text{-}SvO_2))/100$
BE(ecf)	$HCO_3^-(act) - 24.8 + 16.2(pH - 7.40)$
BE(B)	$(1 - 0.014 \times Hb)[(HCO_3^-(act) - 24.8) + (1.43 \times Hb + 7.7)(pH - 7.40)]$

PvCO2: venous partial pressure of carbon dioxide (mmHg); Hb: haemoglobin concentration (g dL^{-1}); SvO2: venous oxygen haemoglobin saturation of oxygen (%).

The simplest definition of BE is the amount of acid required to restore one litre of blood to its normal pH (7.4) when the PCO_2 is kept at 40 mmHg. The normal reference interval of BE for cats is 0 to -5 mmol L^{-1} (Hopper et al. 2014a). The BE will be a more negative number than the reference interval if there is a metabolic acidosis (indicating acid should be removed rather than added), and will be a more positive number than the reference interval if there is a metabolic alkalosis, indicating that acid should be added.

The AG is the mathematical difference between the sum of the routinely measured cations (sodium: Na^+; and potassium: K^+) and anions (chloride: Cl^-; and bicarbonate ion: HCO_3^-) of blood (all mmol L^{-1}) and has been expressed in the following equation (Hopper et al. 2014a):

$$AG = (Na^+ + K^+) - (Cl^- + HCO_3^-)$$

The law of electrochemical neutrality is applied to the concept of AG; this law states that blood is electrochemically neutral and that the sum of cations must always equal the sum of anions (Emmett & Nairns 1977). However, in clinical practice, all known cations and anions in blood are not routinely measure; and therefore the AG suggests that there is a “gap” between the routinely measured cations and anions. In cats, the normal AG ranges from 16 to 20 mmol L^{-1} (Hopper et al. 2014a). If there is an acidosis and the AG is greater than 20 mmol L^{-1} then the cat would have a metabolic acidosis associated

with a wide AG. The wide AG is clinically relevant because it could indicate a build-up of unmeasured acids such as uremic acids and lactic 'acid' for example. A low AG is more of a challenge to determine its clinical relevance and is not well recognised as a useful classifier of metabolic acid-base disturbances. Therefore, in cats, any metabolic acidosis associated with an AG of less than 20 mmol L^{-1} is classified as a metabolic acidosis with normal AG (Hopper et al. 2014a).

The Stewart method suggests that the HCO_3^- and H^+ represent the effect rather than the cause of acid-base derangements (Hopper 2015b). Furthermore, the Stewart method is based on the dissociation of water (H_2O) to produce H^+ or hydroxide ions (OH^-) to maintain electrical neutrality within a solution (like blood) where there are independent variables (arterial partial pressure of carbon dioxide [PCO_2], strong ion difference [SID] and total weak acids [Atot]) and dependent variables (H^+, OH^-, HCO_3^-, CO_3^{2-}, weak acids [HA] and ions [A^-]) which influence the neutrality (Stewart 1978). Any change in the independent variable will create a change in the dependent variables to maintain electrical neutrality within the solution.

Stewart's theory has led to a revised version of the blood pH equation as follows (Constable 2000; Constable 2014):

$$pH = pK_1' + \log\{([SID^+] - Ka[Atot]/(Ka + 10^{-pH})) / S.PCO_2\}$$

where pK_1' is the equilibrium dissociation constant of carbonic acid; *S* is the solubility coefficient of carbon dioxide in plasma; Ka is the effective equilibrium dissociation constant of weak acids, the value is species dependent ($Ka = 0.67 \times 10^{-7}$ where pKa = 7.17; cats [McCullough & Constable 2003]). When using the Stewart method, the veterinarian must measure (PCO_2 using a blood gas analyser) or calculate the independent variables to help interpret the resultant blood pH. Strong ion difference and total weak acid concentrations may be calculated using frequently published equations in the veterinary literature (**Table 1.3**).

Table 1.3 Equations used to calculate the strong ion difference (SID) and total weak acids (Atot) that are required for the Stewart method of acid-base analysis.

Parameter	Formula
SIDapparent	([Na+] + [K+] + [Ca2+]) − [Cl−]
Albumin contribution	Measured albumin × ((0.123 × pH) − 0.631) × 10
Phosphorus contribution	Measured phosphorus × 0.323 × ((0.309 × pH) − 0.469)
Atot	Albumin contribution + Phosphorus contribution
SIDeffective	[HCO3−(act)] + albumin contribution + phosphorus contribution
Strong ion gap	SID apparent − SID effective

Albumin, g dL^{-1}; phosphorus, mg dL^{-1}; electrolytes and lactate, mmol L^{-1}; SID, strong ion difference; Atot, total quantity of weak acids.

The SIDapparent is calculated using routinely measured electrolyte variables and stems from the same logic of electrochemical neutrality and that unmeasured strong ions make up the resultant difference. In cats, the SIDapparent ranges from 40 to 44 mmol L^{-1}. While a SIDapparent greater than 44 mmol L^{-1} correlates with a metabolic alkalosis, a SIDapparent less than 40 mmol L^{-1} correlates with a metabolic acidosis. The SIDeffective is used to estimate the contribution that the weak acids make to the pH. Carbonic acid is a weak acid and dissociates into HCO_3^- which is readily measured, but the albumin and phosphate contributions are more complex to measure and calculate (Hopper 2015b). The strong ion gap (SIG) is calculated by subtracting the SIDeffective from the SIDappraent and this gap assists in identifying if there are unmeasured anions contributing to an acid-base disturbance. Unidentified anions include, but are not limited to ketoacids, sulphate, formic and salicylate acids. If the SIG is greater than 9 mmol L^{-1} then there is an increase in the SIG and a clinical investigation is required to identify the unmeasured acid (Hopper et al. 2014a). Lactate has been included into the SIDapparent calculation (Constable 2000), or excluded, as in this thesis. The logic for including lactate into the SIDapparent calculation is that it is a strong ion (Constable 2000). Others have decided not to include lactate in the SIDapparent calculation, likely because lactate is not a routine analyte measured in blood and if an increase in SIG is identified than lactate and ketoacids should be the first suspected contributors to the SIG (Hopper et al. 2014a).

The Atot is made up of all of the weak acids found in the plasma; the most important constituents are derived from albumin and phosphorus (Hopper 2015a). Their contributions need to be calculated because the blood pH affects their respective level of ionisation or the magnitude of their negative

charge. In cats, an Atot greater than 17 mmol L^{-1} denotes a metabolic acidosis while a value below 8 mmol L^{-1} is classified as a metabolic alkalosis (Hopper et al. 2014a).

The semi-quantitative method requires a number of calculations to determine the effects that albumin, chloride, phosphate, sodium and lactate concentrations have on acid-base balance (Fencl et al. 2000). Because this method considers many processes, it allows for the detection of an acid-base disturbance even when the Henderson-Hasselbalch and Stewart methods do not (Hopper et al. 2014a). The first step is to correct the chloride concentration to determine if the chloride concentration results from changes in free water or because of an acid-base disturbance. Each species of animal has a different normal reference interval for sodium, chloride, phosphorus and albumin, and a mid-range value for the population should ideally be determined. In clinical practice, the mid-range of the in-house laboratory values could be used, or in experimental situations, a similar population of healthy cats should be sampled to determine a normal reference interval (Hopper et al. 2014a). In this thesis, we make use of the health check values to determine the mid-normal value of the study populations reference interval. We made use of published equations already used in veterinary critical care studies to determine the semi-quantitative values (**Table 1.4**).

Table 1.4 Equations used to calculate the effects of the variables responsible for acid-base balance using the semi-quantitative method of acid-base analysis.

Parameter	Formula
Corrected chloride	Measured [Cl−] × (mid-normal [Na+]/measured [Na+])
Free water effect	0.22([Na+] − mid-normal [Na+])
Chloride effect	Mid-normal [Cl−] − corrected [Cl−]
Phosphate effect	0.58 (Mid-normal [phosphorus] − measured [phosphorus])
Albumin effect	3.7 (Mid-normal albumin − measured [albumin])
Lactate effect	−1 × [lactate]
Sum of effects	Free water effect + chloride effect + phosphate effect + albumin effect + lactate effect
Unmeasured ions effect	Base excess − sum of effects

Note: Mid-normal values were determined as the central value of the reference interval of the cats during the health check sampling of blood. Albumin, g dL^{-1}; phosphorus, mg dL^{-1}; electrolytes and lactate, mmol L^{-1}.

The change in effect outside of the recommended reference interval for the species creates an acidosis or alkalosis. Often, more than one of the effects would be outside the normal reference interval, but the diagnostic and clinical relevance of that change is not established. Therefore, despite finding a change known to cause an acid-base disturbance, no treatment recommendations have emanated from these observations compared to the other two acid-base analysis methods. In cats, the various effect reference intervals have been published to indicate an acidosis or alkalosis effect (Hopper et al. 2014a).

When the free water effect is greater than 0.7 mmol L^{-1} then a concentration alkalosis is present; when its effect is less than -1.0 mmol L^{-1} then a dilutional acidosis is present. If the chloride effect is greater than 5.0 mmol L^{-1} then an alkalosis is present; when its effect is less than -4.0 mmol L^{-1} then an acidosis is present. When the phosphorus effect is greater than 1.0 mmol L^{-1} then there is an alkalosis; when it is less than -1.0 mmol L^{-1} then there is an acidosis. The albumin effect is controversial because despite its change in concentration, there are no compensatory mechanisms to correct the albumin effect and therefore its actual contribution to the change in pH is debated (Hopper & Epstein 2012; Ha et al. 2013; Hopper et al. 2014a, b). However, if the albumin effect is greater than 3.0 mmol L^{-1} then an alkalosis is present; if its effect is less than -3.0 mmol L^{-1} then an acidosis is present. The lactate effect can only cause an acidifying effect and this occurs when it is greater than -2.0 mmol L^{-1}. The summation of the effects is subtracted from the BE to determine the unmeasured ion effect; if the resultant of the equation is greater than 0.5 mmol L^{-1} then there are unmeasured alkalis present; if the resultant of the equation is less than -0.5 mmol L^{-1} then there are unmeasured acids present, and an investigation to detect ketoacids is often warranted in critically ill patients.

To date, the field of acid-base balance analysis remains “confusing, irrational and controversial” and there are many strong opinions that remain (Berend 2013). The continued debate on which method is best to use in general, or which method to use in specific disease processes, or how the value of the analysis and its interpretation assists in monitoring and treating disease processes is not standardised. Acid-base disturbances are important clinical findings in animals undergoing general anaesthesia, being managed in intensive care units or requiring critical care; in general, up to 40% of animals will have some sort of acid-base disturbance (Hopper et al. 2014a). In cats, electrolyte disturbances outside of their normal reference interval have been associated with mortality (Goggs et al. 2018). The human medicine literature makes use of a range of different equations to apply the various methods of analysing acid-base disturbances, and the lack of consensus on which equation to use adds to the general confusion. In veterinary medicine, acid-base analysis is less frequently reported compared to human medicine and therefore, there are less applied equations. We standardised our approach to acid-base analysis to conform to equations and methods of analysis that are frequently published by experienced veterinary criticalists (Hopper et al. 2014a), in the hope of contributing to the field of acid-base analysis within our clinical context of cats under general anaesthesia that haemorrhage and require resuscitation.

Haemorrhage, especially if severe, is expected to alter the haemoglobin content within the intravascular compartment (because of less red blood cells) after compensatory fluid shifts occur. Haemoglobin concentration is used to calculate various acid-base variables, as described above, however, this change in haemoglobin concentration's role in acid-base balance has not been investigated in cats. Anaemic cats, because of disease processes such as chronic kidney disease or failure or immune mediated haemolytic anaemia, are acidotic, however, their disease process has been thought to be the aetiology of the acidosis and not the low haemoglobin concentration. An increase in lactate has been noted in critically ill patients that are anaemic, in cats and humans. The increase in lactate arises because of anaerobic metabolism due to oxygen debts that accumulates (Geerken & Gibbons 1972). During haemorrhage, whole blood is lost and therefore the plasma proteins, specifically albumin, also changes in concentration when fluid shifts occur. The role of albumin in blood pH is controversial, however, a decrease in albumin concentration is through to have an alkalising effect, and a decrease is expected to occur, especially when resuscitation fluids are administered (Berend 2013). The change in electrolyte concentrations and its effect on acid-base analysis during haemorrhage and resuscitation is unknown in cats. Haemodilution seems not to play a significant role in acid-base disturbances, but a change in SID, which can occur during fluid administration, does.

During the administration of lactated Ringer's solution or tetrastarch 6% 130/0.4, there is a possibility that the SIDapparent changes, causing some form of an acid-base disturbance, especially if large volumes of fluid are infused. The SIDapparent of lactated Ringer's solution can be calculated using the equation in **Table 1.3** and it is equal to 26.0 mmol L^{-1} (SIDapparent = [131.0 + 5.2 + 1.8] – 112.0). Because there is a relatively wide SIDapparent, these fluids could be considered as being balanced and are theoretically less prone to causing a decrease plasma SIDapparent, even when administered at large volumes. Therefore, these solutions are less prone to causing a SIDapparent related acidosis, or overall acid-base balance (Muir et al. 2011). Tetrastarch 6% 130/0.4 (Voluven) is suspended in physiological saline (0.9% NaCl solution), which is considered an unbalanced isotonic solution. The calculated SIDapparent is equal to 0.0 mmol L^{-1} (SIDapparent = [154.0 + 0.0 + 0.0] – 154.0) and when this fluid is administered at a large enough dose (70 ml kg^{-1} in dogs) it is known to cause an acidosis, specifically a hyperchloraemic acidosis (Langer et al. 2014). Muir (2017) reviewed the effects of administering crystalloid solutions on acid-base balance in domestic animals, however, information related to cats is scantly reported. In dogs, fluid administered in excess altered the haemoglobin and

albumin concentrations but had limited to no effect on blood pH. Muir (2017) concluded that more species-specific research is required to identify the correct fluid selection within the context for which they are prescribed, and that veterinarians cannot easily rely on human medicine data because dogs and cats do not respond in a similar way when fluids are administered.

1.2.10 Haemostasis theory

There are two different models that have been used to explain the theory of haemostasis (coagulation). The first, and older model, is the classic coagulation cascade model and the second is the cell-based model. In the classic coagulation cascade model, haemostasis is divided into two overlapping phases called primary and secondary haemostasis (Hyatt & Brainard 2016). These are also known as the procoagulant pathways, phases or processes. Primary haemostasis is the phase of forming a platelet plug, typically after vascular damage. The secondary phase of haemostasis is where soluble clotting factors are activated to form a fibrin mesh which infiltrates the platelet plug to form a clot. Once the clot has served its function of repairing the blood vessel, it is dissolved through a process called fibrinolysis (Hyatt & Brainard 2016).

The primary phase of haemostasis requires the platelets to be activated; and there are many mechanisms for activating platelets. Damage to the vascular endothelium exposes the subendothelial extracellular matrix which contains von Willebrand factor (vWf) antigen and collagen (Offermanns 2006). The resting circulating platelet will make contact with the subendothelial cellular matrix through the interaction of von Willebrand factor binding to receptors on the platelet called glycoprotein Ib (GPIb-XI-V), as seen in **Figure 1.1**. The glycoprotein VI (GPVI) receptors on the platelet surface bind to collagen as well, and both will tether and activate the platelet. The activated platelet will be stimulated to release a number of signalling mediators (dense granules release serotonin and adenosine triphosphate [ADP]; and alpha granules release platelet-derived growth factor, fibrinogen, vWf) that will be used to attract and activate other platelets to bind to the tethered platelets (Offermanns 2006). This process is called platelet aggregation and is mainly mediated through the integrin beta 3 (specifically, alpha IIb beta 3: αIIbβ3) which binds its ligands vWf and fibrinogen. Platelets will continue to aggregate

and activate in a 'self-perpetuating' cycle until a platelet plug is formed, which is the aim of the primary phase of haemostasis (Hawiger 1987).

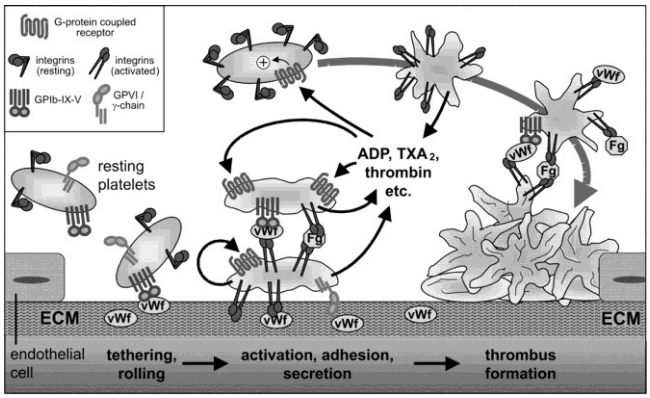


Figure 1.1 The primary phase of haemostasis begins when the blood vessel is damaged, then the subendothelial extracellular matrix (ECM) is exposed. The extracellular matrix has von Willebrand factor (vWf) and collagen which is exposed. The vWf will bind to receptors on the platelet called glycoprotein Ib (GPIb-XI-V) and tether to the ECM. Glycoprotein VI (GPVI) receptors on the platelet surface bind to collagen as well; and both will tether and activate the platelet. The activated platelet will degranulate and release mediations, such as adenosine triphosphate (ADP) which will activate more platelets. Integrins found on activated platelet surfaces, specifically, αIIbβ3 will bind its ligands vWf and fibrinogen (Fg) which causes platelets to aggregate. The cycle of activation and aggregation continues until a platelet plug (thrombus formation) is formed.

Figure taken from: Offermanns S (2006) Activation of platelet function through G protein-coupled receptors. Circ Res 99, 1293-1304.

The secondary phase of haemostasis in the cascade model comprises three pathways: the intrinsic and extrinsic pathways that both culminate into the final common pathway (Badimon et al. 2012). The model relies on the sequential activation of various clotting factors, in response to a stimulation, until the final product of fibrin strands is produced. The intrinsic pathway is where clotting Factors XII, XI, IX and VIII (FXII, FXI, FIX, FVII, respectively) are activated. The extrinsic pathway is where clotting Factor VII (FVII) and tissue factor (TF) are activated. The common pathway is where clotting Factors X, V, II and I (FX, FV, FII and FI, respectively) are activated; this cascade model can be viewed in **Figure 1.2** (Hyatt & Brainard 2016).

The common pathway is essential for the formation of fibrin (Badimon et al. 2012). The intrinsic and extrinsic pathways culminate at a common part where FX is activated to from FXa. The FXa is required to cleave prothrombin (FII) into its active form, thrombin (FIIa), which is a serine protease. Thrombin is then responsible for the activation of fibrinogen (FI) through cleaving it to the active from called soluble fibrin (FIa). Thrombin is also responsible for the activation of FXIII to its active form FXIIIa, which is required to create insoluble fibrin which intercalates with the platelet plug to form a stable clot (Badimon et al. 2012).

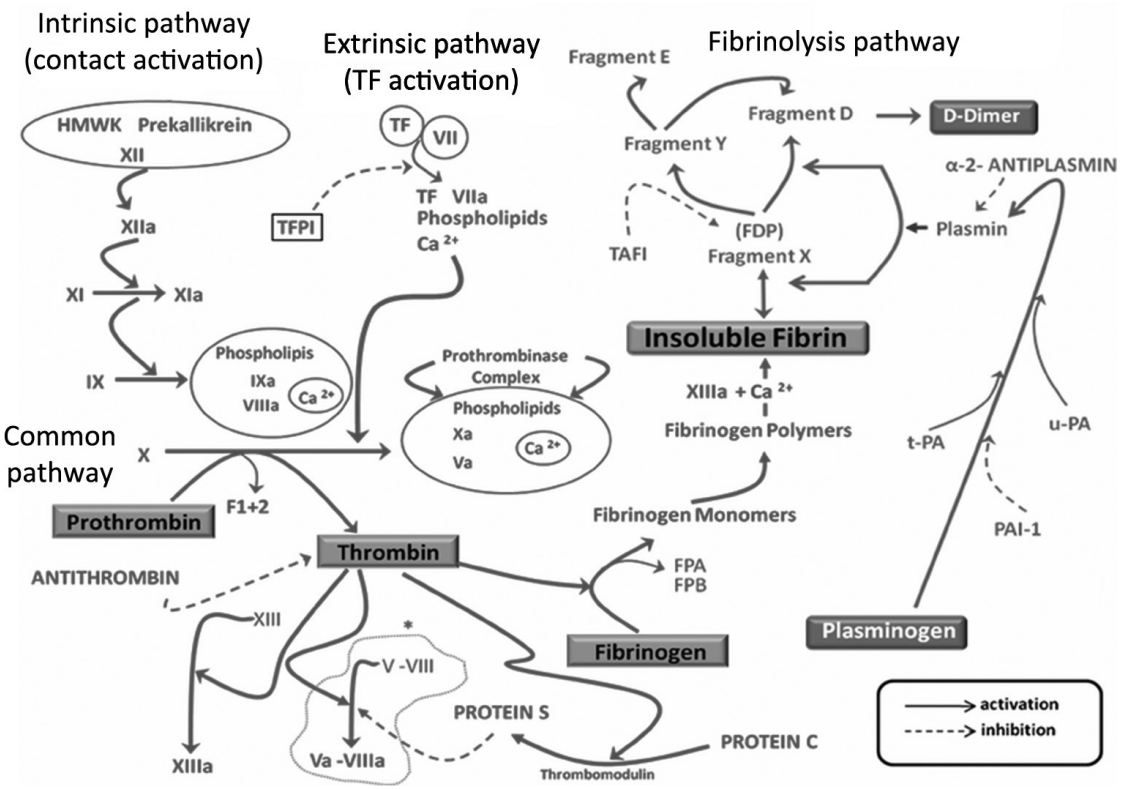


Figure 1.2 The secondary phase of haemostasis depicting using the classic coagulation cascade model. Clotting factors are sequentially activated after an initial activation. The intrinsic and extrinsic cascades both culminate in the common pathway which is the final phase of fibrin formation. The fibrinolysis pathway is where the insoluble fibrin is broken down and dissolved.

Figure adapted from: Badimon et al. (2012) Atherosclerosis, platelets and thrombosis in acute ischemic heart disease. Euro Heart J: Acute Cardiovasc Care 1, 60-74.

The second model of haemostasis is the cell-based model and is claimed to be the most appropriate model to explain coagulation *in vivo* (Smith 2009). In this model, three distinct overlapping phases require the participation of a cell bearing tissue factor and platelets. The reactions occur on the surfaces of the cells (**Figure 1.3**). The phases are initiation, amplification and propagation. When the blood vessel is damaged then the initiation phase begins where circulating plasma FVII binds to TF found on fibroblasts within the subendothelial extracellular matrix (Smith 2009). Once bound, FVII becomes activated (FVIIa) and the TF-FVIIa complex activates FX on the fibroblast surface to form FXa. The TF-FVIIa complex is known as 'extrinsic tenase' (similar to activation of the extrinsic pathway of the classic cascade model) because it activates clotting factor ten. During the initiation phase, the formation of extrinsic tenase is under strong inhibitory control by tissue factor pathway inhibitor (TFPI). It ensures a small amount of locally produced thrombin is formed (Smith 2009). The clotting FXa activates

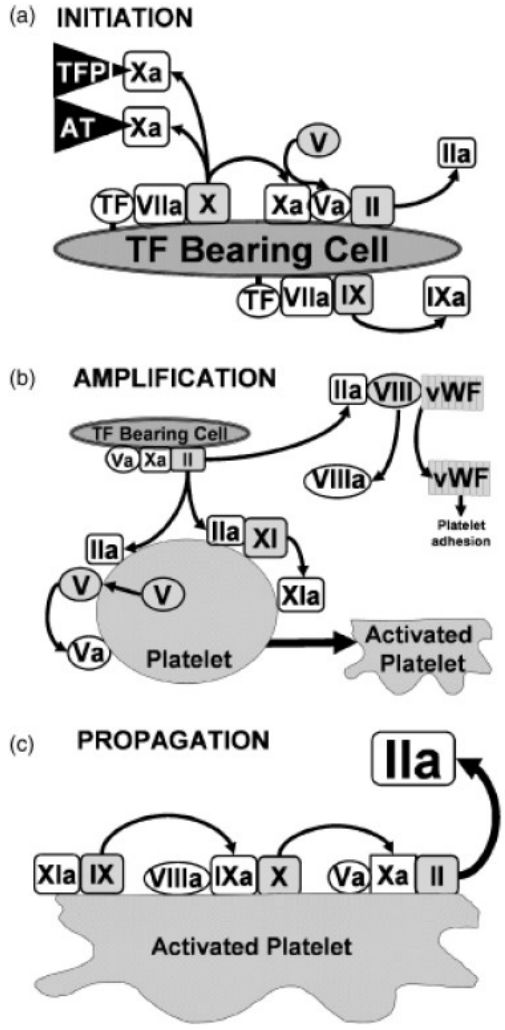


Figure 1.3 A schematic drawing of the cell-based model to explain haemostasis (coagulation). There are three distinct overlapping phases that occur in response to blood vessel damage.

Figure taken from: Smith SA (2009) The cell-based model of coagulation. J Vet Emerg Crit Care 19, 3-10.

prothrombin (FII) to form thrombin (FIIa). The amplification phase commences soon after by the small amount of thrombin (FXa) which amplifies its production on the phosphatidylserine-rich surface of activate platelets. The amplification is achieved by the activating of factors FXI, FV and FVIII. Activated FXI (FXIa) then activates FIX to form active FIXa. Then, FIXa binds to active FVIII (FVIIIa) on the platelet surface to form the 'intrinsic tenase' complex, FIXa-FVIIIa complex. Both, tenase complexes are responsible for the formation of FXa, however, once the factors of the intrinsic and common pathways are activated, they also form active FVa which binds to Xa to create the 'prothombinase complex', FXa-FVa complex (Smith 2009). The FXa-FVa complex is responsible for the amplification and propagation of large amounts of thrombin, the thrombin burst. Thrombin is responsible for cleaving fibrinogen (FI) into active soluble fibrin (FIa). The thrombin burst and the increase in soluble fibrin also stimulate the activation of FXIII. Activated FXIIIa is responsible for cross-linking fibrin to form insoluble fibrin and thus a firm clot. Also, FXIIIa concurrently inhibits the fibrinolysis. Calcium is an essential element, required for all aspects of fibrin formation and allowing coagulation factors to bind to the cell membranes (Smith 2009).

Regardless of the model used to describe haemostasis, there are anti-haemostatic (anti-coagulant) processes that occur at the same time as the active haemostasis to prevent the clot from becoming too large and to prevent systemic-wide coagulation activation. The anti-coagulant system comprises of four independent enzyme pathways that reduce overall thrombin production or limit its creation, or both

(Ezihe-Ejiofor & Hutchinson 2013). The enzyme pathways are 1) antithrombin-glycosaminoglycan, 2) Protein C, 3) TFPI, and the lesser-known, 4) protein Z dependent inhibitor (Ezihe-Ejiofor & Hutchinson 2013). These pathways are activated at the same time as the pro-coagulant pathways, and thrombin, which is generated by the common pathway of the pro-coagulant pathway, has an essential feedback mechanism to stimulate the anti-coagulant pathways. The end product is a platelet plug that has been infused with insoluble fibrin strands that develops into a firm clot (or thrombus). Once the firm clot has achieved its functions of haemostasis and blood vessel repair, then it will be dissolved through the fibrinolysis pathway (Badimon et al. 2012).

Fibrinolysis begins when enzymes called tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA) are released and cleave plasminogen to form active plasmin. Plasmin is a serine protease, its main function is to dissolve insoluble fibrin clots into fibrin degradation products (FDP). To prevent clot breakdown, there are mechanisms in place that inhibit the activation of plasminogen through blocking tPA and uPA by plasminogen activator inhibitor 1 (PAI-1). The PAI-1 is a serine protease inhibitor (serpin) protein (Badimon et al. 2012). Other mechanisms of preventing fibrinolysis include the formation of thrombin activatable fibrinolysis inhibitor (TAFI) and alpha2-antiplasmin (α2-antiplasmin) which inhibits the ability of plasmin to cleave and dissolve the clot (Badimon et al. 2012).

Coagulopathies are known to occur when isotonic crystalloid and synthetic colloid fluids are administered, especially in excess where hypocoagulation (decreased ability to form a stable firm clot) can occur (Albrecht et al. 2016). The principal mechanism of the reported coagulopathies is through haemodilution whereby platelets, clotting factors and co-factors are diluted to such an extent that a stable clot cannot be formed. However, the hydroxyethyl starch synthetic colloids have non-dilutional mechanisms responsible for coagulopathies. In dogs, tetrastarches cause a decrease in vWf concentration and activity, and also an increase in fibrinolysis (hyperfibrinolysis) that last up to 4 hours post-infusion (Gauthier et al. 2015). The effects of tetrastarch 130/0.4 on platelet function in dogs appear contradictory where negative effects on platelet function *in vitro* at a dilution of 1:5.5 with canine whole blood, that corresponds to an *in vivo* bolus of 20 ml kg^{-1} have been reported (Griego-Valles et al. 2017). Counterintuitively, other *in vitro* and *in vivo* studies report no significant effect on platelet function, even at a dilution of 1:3 with canine whole blood, that corresponds to an *in vivo* bolus of 30 ml kg^{-1}; and at an *in vivo* dose of 20 ml kg^{-1} (McBride et al. 2013; McBride et al. 2016). A proposed mechanism for

tetrastarch 130/0.4 negative effects on platelet function is a decrease in expression of integrin αIIbβ3 receptors on platelets. This integrin is necessary for platelet adhesion and aggregation mainly through the binding with fibrinogen (Phillips et al. 1988; Franz et al. 2001), and possibly coating the surface of platelets and thus affects their binding to vWf and fibrinogen (Deusch et al. 2003). The coagulation effects of isotonic crystalloids and hydroxyethyl starches are scantly reported in cats, and the current evidence is derived from an *in vitro* study (Albrecht et al. 2016). When Ringer's acetate and tetrastarch 6% 130/0.42 were mixed with feline fresh whole blood at a ratio of 1:6 (corresponds to an approximate 15 ml kg^{-1} fluid bolus), both fluids impaired haemostasis when measured using rotational thromboelasometry (ROTEM; a viscoelastic measure of haemostatic function). The tetrastarch 6% 130/0.42 caused a greater hypocoagulable effect compared to the Ringer's acetate fluid. However, the magnitude of the effects for both fluids were mostly within the reference interval of the laboratory and therefore the authors suggested that the effects may have limited clinical relevance (Albrecht et al. 2016).

Conventional testing methods of coagulation include determining the prothrombin time (PT) and activated partial thromboplastin time (aPTT) (Hyatt & Brainard 2016). These methods test the functioning of secondary haemostasis and are easily understood using the classic coagulation cascade model whereby the enzymatic activation of one coagulation factor leads to the activation of subsequent factors (Hoffman & Monroe 2001; Riddel et al. 2007). The cascade model comprises three pathways: the intrinsic and extrinsic pathways that both culminate into the final common pathway. The PT measures the activity of the extrinsic pathway (Factor VII and tissue factor) and common pathways (Factors X, V, II and I). The aPTT measures the activity of the intrinsic (Factors XII, XI, IX and VIII) and common pathways. These conventional testing methods are important for clinical practice, but are limited to testing secondary haemostasis only, and therefore viscoelastic coagulation testing methods have been introduced which assess global coagulation function (Brooks & Catalfamo 2013; Hyatt & Brainard 2016).

Thromboelastography (TEG) is one of the viscoelastic coagulation testing methods and has been used to assess global coagulation function in cats (Alwood et al. 2004; Donahue & Otto 2005; McMichael & Smith 2011; Cuq et al. 2017). When running this test, a tracing is plotted at 2 mm minute^{-1}, called the thromboelastogram, which consists of three phases (**Figure 1.4**).

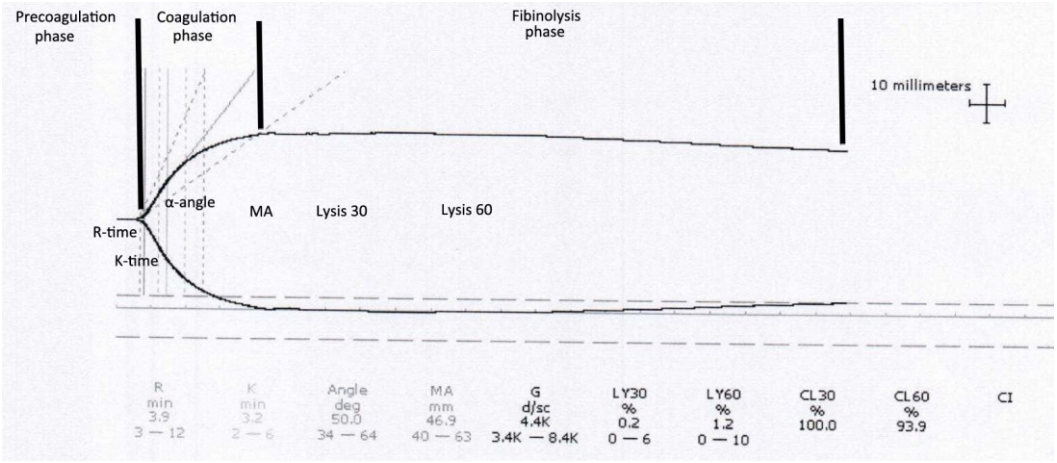


Figure 1.4 The thromboelastogram tracing consists of three phases which represent the different phases of coagulation. The precoagulation phase starts from the initiation of the test and ends when the first few fibrin stands are formed. The coagulation phase assesses how large a clot can become and starts after the first few stands are formed and ends when the maximum amplitude is reached (MA). The fibrinolysis phase assesses how quickly the clot breaks down and starts after the MA until the end of the test time, usually 120 minutes.

Figure taken from the present study data set from the health check examination and adapted by Zeiler GE.

The phases are 1) precoagulation phase which is from the initiation of the test until the first few fibrin stands are formed; 2) the coagulation phase which assesses how large a clot can become; and 3) the fibrinolysis phase which assesses how quickly the clot breaks down (Donahue & Otto 2005). Many measurements can be made from the tracing, and these are used to evaluate the global function of coagulation (Donahue & Otto 2005; Hyatt & Brainard 2016). The reaction time (R-time) is the time (in minutes) from starting the test until the lines of the tracing diverge by 1 mm. The R-time is influenced by Factor XII, XI, IX and VIII and thus evaluates the activity of the intrinsic pathway. The kinetic time, or clot formation time (K-time) is the time from the end of R-time until the lines diverge by 20 mm. K-time is influenced by Factors II (prothrombin) and VII, platelets, thrombin (Factor IIa) formation, fibrinogen (Factor I) concentration and haematocrit. The maximum amplitude (MA) is the maximum distance between the diverging lines and is measured in mm. The MA reflects clot strength and is influenced by fibrin (Factor Ia), fibrinogen (Factor I) concentration, platelet count, Factor XIII and haematocrit. The alpha-angle (α-angle) is the angle between the midline and the tangent to the curve from the 1 mm divergence point and is a measure of the speed of clot formation, similar to K-time (Donahue & Otto

2005). Lysis 30 and Lysis 60 are the distances between the divergent lines at 30 and 60 minutes after reaching MA. They are represented as a percentage of MA. They are an indication of how quickly the clot is undergoing fibrinolysis and thus, overall clot stability (Hyatt & Brainard 2016).

1.2.11 Conclusions

Considering the haemorrhage research models and the physiology of haemorrhage in cats it becomes clear that there are a number of concerns that need to be accounted for when planning a meaningful study model and design. These concerns include:

1) Cats have a very varied and individualised response to haemorrhage, where the volume of blood removed does not always translate into a reliable decrease in blood pressure.
2) The volume and, more importantly, the rate of blood removal should be standardised.
3) Cats are known to reach a critical hypotensive point (MAP between 40 and 60 mmHg) where, if enough volume is withdrawn, they will succumb and die rapidly. The reason for the rapid death once a critical blood volume has been lost is because they have a profound compensatory response to haemorrhage which maintains their blood pressure. This compensation is achieved through vasoconstriction and the recruitment of blood from the splanchnic vascular bed. Once these compensatory responses are exhausted, then the cat succumbs.
4) All of the researches where in cats were experimentally haemorrhaged were terminal studies and cats were sacrificed at the end of the study data collection period if they did not die earlier during the experimental procedures.
5) The cats were anaesthetised during the haemorrhage and other experimental procedures.
6) Haemorrhage-induced hypotension alters the physiology of the cardiovascular, respiratory, central nervous and, endocrine systems as well as the physiology of the metabolism, the gastrointestinal tract and other splanchnic organs. Very little to no investigation has been conducted on the blood acid-base status and coagulation in cats. However, during haemorrhage and resuscitation, the cardiovascular and respiratory systems are perhaps the most critical systems to monitor. If these systems are able to compensate, then it is assumed

that cerebral blood flow and other organs perfusion will be maintained within limits to avoid post-experimental procedure complications despite a presumed low cardiac output.

We aimed to complete a study using cats as a live animal model where, while being anaesthetised and haemorrhaged, two different resuscitation fluids were administered. The study design was a random allocated crossover design were the cats underwent three treatments. Therefore, we had to avoid death in the cats, especially during the haemorrhage phase of the study. From the information already gained we were able to provide endpoints of the haemorrhage stage of the research, and because of the individualised response to haemorrhage we aimed to use a hybrid model where we used a fixed-volume and fixed-pressure model as endpoints. Furthermore, we did not investigate the effects of haemorrhagic shock, therefore immediately after haemorrhage the cats were administered the resuscitation fluid. We made the following assumptions and defined the following endpoints. We assumed the cat's total blood volume to be 55 mL kg^{-1} and a severe haemorrhage event to be an acute blood loss of more than 22 mL kg^{-1} (> 40% assumed circulating volume). The blood was withdrawn manually at a targeted rate of 2 mL kg^{-1} per minute (over 15 minutes) until one of two endpoints. The endpoint was either a maximum withdrawal of 30 mL kg^{-1} of blood; or a MAP of < 48 mmHg that persisted for at least 3 minutes. The volume replacement rates will be done over 120 minutes by a controlled set intravenous infusion of the resuscitation fluids. The estimated blood loss was 30 ml kg^{-1} over the entire data collection period (haemorrhage phase and blood sampling during the resuscitation phase). We also planned to administer large resuscitation volume to create a fluid overload to identify at what volume should fluids be stopped. We used 4:1 ratio for the lactated Ringer's solution and administered at 60 ml kg^{-1} per hour for 120 minutes (total dose 120 ml kg^{-1}); and we used a 1.5:1 ratio for the tetrastarch 6% 130/0.4 at 20 ml kg^{-1} per hour for 120 minutes (total dose 40 ml kg^{-1}). The ratio dose rates were similar to the recommended shock dose fluid rates.

Another concern raised was that the general anaesthesia could alter the true physiological effects of haemorrhage and resuscitation. We anaesthetised the cats during the study and had to also standardise the anaesthetic and analgesic protocol. We proposed that, within the context of our study, we had to design it in such a way that the information would be as transferable as possible from the bench to practice, and therefore we made use of what we believe to be a cardiovascular-sparing drug combination that included an opioid, alfaxalone and isoflurane. Similar drug combinations have been advocated for routine or emergency surgery. Furthermore, the literature review highlights the lack of

progress in 1) determining novel methods of quantifying acute blood loss to better identify the transfusion trigger, and 2) understanding fluid management in cats with regards to the resuscitation phase whereby cats are claimed to be fluid-sensitive and require very conservative fluid therapy. Whether that is true in healthy anaesthetised cats that have undergone severe haemorrhage-induced hypovolaemic hypotension is not known. This lack of evidence is resonated by many authors, over the recent few decades, and they make claim that further research that can be used in to improve our management of cats in clinical practice is desperately required (Hughes 2001; Prittie 2006; Driessen & Brainard 2006; Hammond & Holm 2009; Silverstein et al. 2012; Mazzaferro & Powell 2013; Silverstein et al. 2014; Glover et al. 2014; Boller & Boller 2015; Muir et al. 2017; Ostroski et al. 2017; Adamik & Yozova 2019).

1.3 Scope of the thesis

1.3.1 Problem statement

Surgical procedures have advanced in cats but the risk of intraoperative haemorrhage is always present. Most healthy patients can tolerate an acute blood loss of 10% of their blood volume without warranting volume resuscitation. However, there is a lack of insight to predict or quantify severe haemorrhage, and there is an underpreparedness to treat severe haemorrhage using commonly prescribed resuscitation fluids in cats. Currently, most of the recommendations are anecdotal, gained from clinical experience over the last few decades, or extrapolated and adjusted from dog-derived evidence. Evidence-based outcomes on rapid fluid administration to correct life-threatening haemorrhage-induced hypovolaemia and hypotension in anaesthetised cats are lacking in the literature.

1.3.2 Aims of the thesis

Cats are common pets all over the world and we need to improve the clinical management of these animals while anaesthetised and undergoing surgery. Cats are more likely to die during the anaesthetic recovery period than at other times, and fluid management has been highlighted as a risk factor that could contribute to their death. The presence of intraoperative haemorrhage can often be controlled by the surgeon. Therefore, a controlled haemorrhage research model in cats is a valid model to investigate haemorrhage and resuscitation and our findings can translate into real clinical practice. This thesis focused on investigating sequential aspects of acute severe haemorrhage and fluid administration to treat hypovolaemic-hypotension in anaesthetised cats, but in particular 1) the physiological response to an acute severe haemorrhage and 2) the physiological response to fluid administration to treat haemorrhage-induced hypovolaemic hypotension. Below is an outline of how each subsequent chapter furthers our understanding of severe haemorrhage and fluid resuscitation in anaesthetised cats. Chapter 2 address the deficiencies in the literature by describing the extensive physiological effects of acute severe haemorrhage. Chapters 3, 4 and 5 address the deficiencies in the literature by describing the extensive physiological, acid-base and haemostatic effects of fluid administration to treat haemorrhage-induced hypovolaemic hypotension.

Chapter 2: Development of a severity scoring system for acute haemorrhage in anaesthetised domestic cats: The CABSS score

In human medicine, there are scoring systems used to score a patient's physiological response to blood loss. These systems are used in pre-hospital trauma or in-hospital or in-operating room scenarios where a patient that has lost blood is scored to determine if a transfusion is required (Pons et al. 1985; Baskett 1990; Yucel et al. 2006; Chico-Fernandez et al. 2011; Ogura et al. 2014; Callcut et al. 2016). Surgical procedures have advanced in cats but the risk of intraoperative haemorrhage is always present (Bellenger et al. 1996; DeLay 2016; Hanson et al. 2017). To date, there are no haemorrhage-related scoring systems in veterinary medicine which aid in the decision-making of whether an animal requires a transfusion or not. However, in veterinary trauma medicine, the abdominal fluid score obtained during an abdominal ultrasound examination is a scoring system designed to detect and monitor fluid production within the abdominal cavity. Typically, during the ultrasound examination, a fluid sample is collected by abdominocentesis and is analysed. If the fluid is whole blood (similar to a venous blood sample), then the patient is monitored by repeating the scans at set time intervals to determine if haemorrhaging is uncontrolled and active. Other scoring systems used in veterinary medicine are not specific to haemorrhage and the physiological effects of haemorrhage to aid in decision-making of transfusion requirements. Blood loss during a surgical procedure is estimated, because no accurate method is available to quantify blood loss. Determining the volume in suction bottles, counting quantity of blood-soaked swabs and estimating blood loss on drapes and around the surgical area are used to estimate external blood loss, but quantifying internal blood loss is not easy to do (Jutkowitz 2004). Furthermore, each cat copes differently to set losses of blood volume, therefore just quantifying a blood loss volume is not a very precise method of determining if the animal requires a blood transfusion. Cats are known to have a profound compensatory mechanism to large volumes of blood loss whereby they will maintain a fairly normal blood pressure despite being volume-depleted until near cardiovascular collapse (Barcroft et al. 1925; Barcroft & Stephens 1927; Greenway & Stark 1969; Lautt et al. 1980; Breznock & Strack 1982). This physiological capacity makes determining the severity of the physiological effects of blood loss even more challenging, especially when anaesthetised. Therefore, in this investigation, we aimed to investigate the effects of haemorrhage in anaesthetised cats to determine if there are variables that could be used to compile a confidence scoring system to quantify acute blood loss that could be used in clinical practice. To determine the effects we collected many

physiological, haematological and biochemical variables before and immediately after a mild and a severe haemorrhage event. Then the best variables that changed in value before and immediately after haemorrhage were analysed further to determine their suitability for inclusion into a Cat Acute Bleeding Scoring System (CABSS).

Chapter 3: Biomarkers that indicate an endpoint for administering lactated Ringer's solution or 6% tetrastarch 130/0.4 fluids to resuscitate haemorrhage-induced hypovolaemic anaesthetised cats: a concept of volume-endpoints to prevent iatrogenic fluid overload

Administration of isotonic crystalloid or synthetic colloid fluids to resuscitate the intravascular compartment has been used in human and veterinary science. However, each species appears to respond to fluid administration differently and humans are more prone to developing fluid overload where too much fluid is administered, or where the fluid shifts among the compartments in an unpredictable manner (Malbrain et al. 2018). Cats are also claimed to be more fluid-sensitive (similar to humans) compared to dogs (Brodbelt et al. 2007). To date, a range of biomarkers have been suggested to cue the endpoint of the resuscitation phase of fluid administration in hypovolaemic patients or animals. These biomarkers either focus on the cardiovascular effects or on the ability of the blood to transport oxygenated blood to the metabolising tissues to prevent a shock state (Prittie 2006; Boyd & Smart 2018). In controlled bleeding hypovolaemia, liberal and modified fluid administration protocols have been developed in the hope of resuscitating the intravascular compartment effectively without causing fluid overload and associated sequela (anaemia, hypercoagulation, global tissue oedema) (Mazzaferro 2008; Ostroski et al. 2017). Fluids are administered in a goal-directed manner whereby targeted cardiovascular or oxygenation endpoints are predetermined, and the animal receives fluids until the desired reference interval is met (Thomovsky et al. 2016). Currently, the protocol for fluid administration in veterinary emergency medicine is that the shock dose (one circulating blood volume of a healthy animal) of isotonic crystalloid fluids has to be administered in divided doses over time while the animal's response is monitored to determine the effectiveness of the administered fluid. Similarly, the synthetic colloids is also administered in small volumes and titrated to clinical effect. However, there are two concerns: 1) despite this proposed fluid administration protocol, fluid overload and sequela commonly develop, especially in trauma or moribund animals (Mazzaferro 2008), and 2) it is not clear

to the veterinarian when to stop administering fluids when the shock dose has been administered to an animal that has not met the resuscitation biomarker endpoints. In this event, if the animal is not considered as a fluid responder, it should receive a hybrid approach of fluids and catecholamine drug support. The resuscitation endpoints aim to restore the physiological function of the animal as quickly as possible (Prittie 2006), but the risk of administering too much volume is always present, especially in presumed fluid-sensitive animals like cats. Furthermore, administering fluids to an animal that has already sustained severe haemorrhage is somewhat controversial in that fluid overload is perceived as being more likely to occur, and the clinical effects of creating an acute isovolaemic haemodilution state (administering fluids at a volume to replace the blood volume that has been lost) are mostly unknown in cats (Mazzafero 2008; Boyd & Smart 2018). Therefore, instead of resuscitation endpoints, perhaps volume endpoints should be determined, which can be more effective in guiding fluid therapy but also prompting the veterinarian to switch to a hybrid resuscitation approach to avoid accidental idiopathic fluid overload and sequela. Therefore, in this investigation, we aimed to determine if there are volume endpoints that are novel and do not necessarily include traditional cardiovascular and oxygenation based endpoints of resuscitation. To determine if volume endpoints can be defined, cats underwent acute severe haemorrhage followed by fluid administration at a set infusion rate using an isotonic crystalloid or synthetic colloid fluid. During the procedure, many physiological, haematological and biochemical variables were determined. These variables were analysed over time to determine if they changed in a predictable manner during the period of fluid administration. Variables that demonstrated a reliable change in value over time during fluid administration were analysed further to determine if they could become volume-endpoint biomarkers that could be useful during fluid resuscitation in haemorrhage-induced hypovolaemic hypotensive anaesthetised cats.

Chapter 4: Blood acid-base analysis during crystalloid and colloid fluid resuscitation in haemorrhage-induced hypovolemic hypotensive anaesthetised cats

Many physiological processes rely on the activity of enzymes to complete biochemical processes of metabolism and cellular function. In mammals, the activity of these enzymes are pH-dependent, and operative most effectively when the blood pH is between 7.35 and 7.45 (Mitchell et al. 1972; Crimi et al. 2012). Many methods are used to analyse pH. For the purpose of this thesis, we make use of three

methods that have been previously published for use in cats to allow a comprehensive analysis of the blood acid-base balance. The three methods are: 1) the Henderson-Hasselbalch, 2) the Stewart, and 3) the semi-quantitative method (Hopper et al. 2014a, b). These methods include variables like haemoglobin and electrolyte concentrations that are expected to change in when the cats undergo haemorrhage and fluid resuscitation, possibly altering the blood pH. Furthermore, there are concerns about administering balanced versus unbalanced fluid solutions. In normovolaemic animals, the administration of these different fluids can cause changes in the strong ion difference which could alter the blood pH, especially when administered at large volumes (Muir 2017). The dilutional effects appear to have a minor contribution. However, during severe haemorrhage and fluid resuscitation in cats, the effects of dilution might contribute more compared to the normovolaemic animals. There is a paucity of data in the literature detailing the blood pH response to haemorrhage and fluid resuscitation in anaesthetised cats. In order to address this lack of information in the literature, we aimed to collect all the variables required to analyse and report the blood pH status of the anaesthetised cats over time during haemorrhage and fluid resuscitation using the three methods of analysis. We also aimed to determine if the balanced isotonic crystalloid causes a different effect on pH compared to the unbalanced isotonic fluid that suspends the synthetic colloid fluid (6%, 130/0.4).

Chapter 5: Effects on coagulation during fluid resuscitation using lactated Ringer's solution or 6% tetrastarch 130/0.4 in controlled haemorrhaged anaesthetised cats

In dogs, *in vitro* and *in vivo* investigations found that all fluids, if given in large enough volumes altered haemostasis (Driessen & Brainard 2006; Brooks et al. 2014; Adamik et al. 2015; Wurlod et al. 2015). A single *in vitro* investigation in cats could be found and they reported that haemostasis changed when blood was diluted; but it's clinical relevance was questioned (Albrecht et al. 2016). These investigations also included a severe haemorrhage component to the study with administration of fluids to correct the hypovolaemia. Furthermore, the hydroxyethyl starch colloid solutions are known to cause non-dilutional effects that alter platelet function and adhesion, which causes further hypocoagulability (Adamik et al. 2015). If administering resuscitation fluids alters haemostasis then this can complicate the control of intraoperative haemorrhage, especially if there is insidious seeping from the vascular tissues. Furthermore, a stable clot has a vital function of tissue repair, and if there is hypocoagulability, this

could delay post-surgical convalescence (Niles 1999). We speculated that a similar effect on homeostasis might occur in anaesthetised cats undergoing severe haemorrhage followed by fluid resuscitation. However, there are scant reports on haemostatic effects of fluid administration in cats. To address the lack of evidence, we aimed to collect blood samples to analyse haemostatic function using traditional and global clotting viscoelastic assays to determine the effects of haemorrhage followed by fluid administration in anaesthetised cats.

The series of studies presented in this thesis were approved by the University of Pretoria Faculty of Veterinary Science's Research Committee and Animal Ethics Committee (v006-15), and the Animal Ethics Research Committee of the University of Witwatersrand (2017-10-68-C-AREC) prior to commencement of the investigation.

CHAPTER 2

Development of a severity scoring system for acute haemorrhage in anaesthetised domestic cats: The CABSS score

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2.1 Abstract

Objective To determine if there are physiological, haematological, biochemical or electrolyte variables that can be used to predict severe haemorrhage in cats.

Study design Randomised crossover study whereby each cat underwent mild and severe haemorrhage, with a 2-month period between events.

Animals Six domestic cats (21 ± 1 months old; 4.9 ± 1.2 kg).

Methods The cats were anaesthetised (buprenorphine, alfaxalone, isoflurane in oxygen, fixed end-tidal concentration of 1.7%) before the haemorrhage event. A total of 34 variables were measured twice (pre-haemorrhage and post-haemorrhage). The difference and percent change for each variable were compared between haemorrhage events (paired t-test). Significant variables were placed into 13 different ratios (post-haemorrhage value of one variable divided by a post-haemorrhage value of a second variable) and compared (paired t-test) and Cohen's *d* (*d*) was calculated. Receiver operating characteristic curves were plotted and cut-off values for weak, moderate and strong indicators of severe haemorrhage were obtained.

Results The blood loss was 4.5 ± 1.1 mL kg^{-1} and 26.8 ± 5.5 mL kg^{-1} for the mild and severe haemorrhage events, respectively. The most significant variables with large effect-size were heart rate, systolic arterial blood pressure, end-tidal carbon dioxide, serum albumin, haematocrit and actual bicarbonate ion concentration. The most robust ratios were the 1) shock index (*d* = -2.8; heart rate: systolic arterial blood pressure), 2) heart rate: end-tidal carbon dioxide (*d* = -2.9), 3) serum albumin: haematocrit (*d* = 1.5), and 4) heart rate: actual bicarbonate ion concentration (*d* = -1.6). These ratios were included in the final proposed Cat Acute Bleeding Scoring System (CABSS).

Conclusion and clinical relevance Cats subjected to mild and severe haemorrhage demonstrated statistically and clinically relevant changes whereby four ratios could be created to make up the CABSS. The ratios detected and quantified the presence of severe haemorrhage in anaesthetised cats.

2.2 Introduction

Life-threatening haemorrhage is when more than 40% of the total circulating blood volume is lost acutely (Mylankal & Wyatt 2013). In veterinary patients, once 30% or more of blood volume has been lost acutely then compensatory mechanisms may fail and signs of haemorrhagic shock develop (Jutkowitz 2004). The mortality rate in domestic cats as a consequence of such haemorrhage is unknown; in humans, unexpected and uncontrolled intraoperative haemorrhage increased the mortality rate from less than 1% to over 20% (Copeland et al. 1991).

Surgical procedures have advanced in cats but the risk of intraoperative haemorrhage is always present (Bellenger et al. 1996; DeLay 2016; Hanson et al. 2017). Most healthy patients can tolerate an acute blood loss of 10% of their blood volume without warranting volume resuscitation. There are several methods used to estimate the volume of intraoperative blood loss, which include monitoring the amount of blood in suction canisters, counting of blood-soaked swabs (sponges) and estimating blood volume loss on surgical drapes (Jutkowitz 2004). Indirect methods used to assess haemorrhage include monitoring the haemoglobin (or haematocrit), albumin or total serum solids (Jutkowitz 2004). However, these indirect methods are only useful to assess blood loss after compensatory fluid shifts have taken place, which is at least 2 hours following an acute haemorrhage event (Jutkowitz 2004). As a result, volume resuscitation in cats experiencing severe haemorrhage may be delayed. Furthermore, the relatively small blood volume of healthy cats, which ranges from 52.6 ± 6.8 to 59.6 ± 5.8 mL kg^{-1}, makes determining overall blood loss a challenge (Groom et al. 1965; Mott 1968). If there is insidious bleeding within the thorax and peritoneal cavities, masked by overlying organs or when the field of vision is limited (thoracoscopy and laparoscopy) intraoperative haemorrhage is likely to be under-recognised in cats and become a possible risk factor for many of the reported cardiovascular-related perianaesthetic deaths (Brodbelt 2010).

To date, there are no scoring systems used to aid in detecting or quantifying acute haemorrhage in awake or anaesthetised companion animals (Reineke 2018), but they are commonplace in human medicine (Pons et al. 1985; Baskett 1990; Yucel et al. 2006; Chico-Fernandez et al. 2011; Ogura et al. 2014; Callcut et al. 2016). The scoring systems applied in human medicine are used to identify patients in haemorrhagic shock, guide resuscitation or as an early transfusion trigger, often before presenting to a hospital (Terceros-Almanza et al. 2019). The ideal scoring system should be one that (1) is easy

to calculate, (2) makes use of physiological variables that reflect an early response to haemorrhage, (3) includes variables that are reflected by changes in the blood, and (4) is composed only of variables that are obtainable at a single time point once acute haemorrhage is suspected.

The aim of the present study was to determine if there are any immediately quantifiable physiological, haematological, biochemical or electrolyte variables that can be used in the Cat Acute Bleeding Scoring System (CABSS) to predict an acute severe haemorrhage event in domestic cats. We hypothesised that none of the variable values obtained before a mild and severe haemorrhage event will be different compared to after the event in anaesthetised cats.

2.3 Materials and Methods

2.3.1 Study design, animals and housing

A group of six domestic cats (mean ± standard deviation, age 21 ± 1 months old, mass 4.9 ± 1.2 kg) were used, based on availability, in this balanced randomised crossover study, where the interventions were mild and severe acute haemorrhage while under anaesthesia, with a 2-month period between events. The order of treatments was randomised using an online randomiser using a balanced single block design (www.randomization.com; Dallal GE; Date last modified: 16 July 2008). The cats were sourced from various places as kittens and were housed together in the University of Pretoria Biomedical Research Centre's indoor-outdoor purpose-built cattery until they were grown enough (weight > 3 kg) to be used in the study. The study was approved by the Ethics Committees of the University of Pretoria (v006-15) and University of Witwatersrand (2017-10-68-C-AREC). This study was part of a larger fluid resuscitation project and only data relevant to this present study are reported.

A clinical examination, that included venous blood sampling from the jugular vein for blood analysis (haematology, albumin, blood urea nitrogen, creatinine, electrolytes), was performed 1-week prior to the haemorrhage event to determine the health status of the cat.

2.3.2 Haemorrhage event procedures

Pre-haemorrhage period

On the morning of data collection, the cat was transferred to the theatre complex of the hospital and placed in a standard ward cage. The cat was weighed and underwent a clinical examination and was premedicated with buprenorphine hydrochloride (0.02 mg kg^{-1}; Temgesic 0.3 mg mL^{-1}; Beckitt Benckiser Healthcare; South Africa) intramuscularly. The cat was transferred to the induction room 45 minutes later and an indwelling catheter (22 Gauge; Jelco; Smiths Medical International; Lancashire, UK) was inserted aseptically into one of the cephalic veins and secured in place. General anaesthesia was induced with alfaxalone (Alfaxalone; Afrivet; South Africa) intravenously, to effect. A cuffed polyvinylchloride endotracheal tube (4.0 to 4.5 mm internal diameter; Teleflex Incorporated; South Africa) was placed into the trachea after a single spray of lidocaine (Xylocaine; AstraZeneca Pharmaceuticals; South Africa) onto the *rima glottidis*. The endotracheal tube was then connected to a circle breathing system (15 mm internal diameter, Compact paediatric breathing system; Intersurgical; South Africa). The cat was maintained under general anaesthesia using isoflurane (Isofor; Safeline Pharmaceuticals; South Africa) in oxygen at a fixed fresh gas flow rate of 80 mL kg^{-1} $minute^{-1}$. The vaporiser was initially set to 2%, target end-tidal isoflurane (FE′Iso) concentration was standardized for the study and maintained at 1.7% throughout the procedures (Shaughnessy & Hofmeister 2014). The cat was placed in dorsal recumbency and its ventral neck region (chin to the second intercostal space of the thorax) was shaved and surgically scrubbed. The cat was transferred to the theatre, placed in dorsal recumbency and instrumented.

A catheter (22 Gauge, 50 mm, Arrow arterial catheterization set; Arrow International; PA, USA) was inserted into the right pre-superficialised (superficialised 15 months earlier during gonadectomy) carotid artery using a cut-down technique to facilitate measurement of direct arterial blood pressure (electronic transducer zeroed to atmospheric air pressure at the level of the right atrium) and intermittent sampling for arterial blood samples for blood gas analyses. A catheter (22 Gauge, 50 mm, Arrow arterial catheterization set) was inserted percutaneously into the left jugular vein for intermittent blood sampling. The Seldinger technique was used to insert the catheters into the carotid artery and jugular vein (Seldinger 1953). A three-lead electrocardiograph (ECG) was assessed by attaching ECG pads and

electrodes placed on the paws (RA, LA, RL; lead II) to assess heart rhythm and rate. A paediatric pitot spirometer with an in-built gas sampling line was attached between the endotracheal tube and breathing system to measure expiratory tidal volumes (VTexp), respiratory rate (f_R) and respiratory gas tensions including end-tidal carbon dioxide ($PE'CO_2$) and FE′Iso. A transmittance pulse-oximetry probe was placed on the tongue to measure peripheral oxygen-haemoglobin saturation (SpO_2). A thermistor probe was inserted into the oesophagus to a depth of the fourth intercostal space.

All probe leads were connected to a multiparameter monitor (Cardiocap 5; Datex; Finland). A balanced electrolyte crystalloid fluid (lactated Ringer's solution; Fresenius Kabi; South Africa) was infused at a constant rate of 5 mL kg^{-1} $hour^{-1}$ throughout the procedures via the cephalic cannula.

Haemorrhage period

Pre-haemorrhage data were collected before the cat underwent the assigned haemorrhage event (24 ± 6 minutes after induction of anaesthesia). After the haemorrhage event was complete, post-haemorrhage data were collected immediately. The entire process of collecting data and completing the haemorrhage event was timed (haemorrhage time). Pre- and post-haemorrhage event data collection included physiological, haematological, biochemical, electrolyte and blood gas variables. The order of data collection was standardised and began with all blood sampling followed by the recording of physiological variables.

The physiological variables were heart rate (HR), arterial blood pressures [systolic (SAP), mean (MAP) and diastolic (DAP)], SpO_2, f_R, V_{Texp}, $PE'CO_2$, FE´Iso and body temperature (T). The haematological variables were differentiated cell counts, haemoglobin concentration (Hb) and haematocrit (Ht) measured from venous blood sample (Ethylenediaminetetraacetic acid collection tube; BD, Becton Dickinson and Company; Plymouth, UK). The biochemical variables were serum albumin, blood urea nitrogen, creatinine, glucose and lactate, measured from venous blood sample (serum clot activator tube; BD). The electrolyte variables were sodium, potassium, ionised calcium, total magnesium, chloride and phosphorus, measured from venous blood samples (blood gas and serum clot activator tube). The blood gas variables were partial pressure of oxygen (PaO_2) and carbon dioxide ($PaCO_2$; $PvCO_2$) and acid-base balance (pH, bicarbonate ion, base excess) measured from arterial and

venous blood samples (pre-heparinised syringes). The total amount of blood drawn per data collection was 10 mL (3 mL waste and 7 mL samples).

The mild haemorrhage event was defined as blood loss sustained during blood sampling which amounted to 20 mL in total, regardless of the cat's body mass. The post-haemorrhage blood sample was obtained 15 minutes after the pre-haemorrhage blood sample.

The severe haemorrhage event was defined as blood loss sustained during purposeful collection of blood into a semi-closed system using citrate-phosphate-dextrose (4 mL; JMS blood bag, 450 mL; JMS Singapore PTE LTD; Singapore) primed 20 mL syringes via the jugular catheter. We assumed the cat's total blood volume to be 55 mL kg^{-1} and a severe haemorrhage event to be an acute blood loss of more than 22 mL kg^{-1} (> 40% assumed circulating volume). Manual blood withdrawal continued at a targeted rate of 2 mL kg^{-1} $minute^{-1}$ (15 minutes) until one of two endpoints. The endpoint was either a maximum withdrawal of 30 mL kg^{-1} of blood, that did not include the blood sampling volume; or a MAP of < 48 mmHg that persisted for at least 3 minutes. The total amount of blood drawn was calculated as a sum of the blood sampling (20 mL) and the purposefully bled volume.

On completion of the haemorrhage event, the cat entered a fluid resuscitation phase of the project that is not reported here. All cats survived and were rehomed through an adoption processes 1 month after completing the project.

2.3.3 Data analysis

Data were assessed for similar distribution types by plotting histograms and comparing descriptive statistics for each variable within the mild and severe haemorrhage data sets. High and low values were tested for being an outlier by using the Grubb's test. Data was considered normally distributed and no outliers were detected. The absolute and percent change from prehaemorrhage values were calculated for all variables. The haemorrhage time, difference and percent change values for each variable were compared between the mild and severe haemorrhage events using a paired t-test. The 95% confidence interval for the mean difference and the *p*-value for each comparison were assessed to initially screen variables to identify those that could be incorporated into the scoring system. This initial screening was supplemented by effect size calculations for each variable (difference and percent change) using Cohen's *d* (*d*). An effect size of $d > 0.8$ was considered large and clinically meaningful (Sullivan & Feinn

2012). All variables that met the initial screening criteria were then placed into various unitless ratios (posthaemorrhage value of one variable divided by a posthaemorrhage value of a second variable) to identify ratios that could potentially be incorporated into the scoring system. The variables included in the ratios were based on published ratios (shock index: Allgower & Burri 1967) or clinical insight and mathematical ease of use. These ratios were calculated for mild and severe haemorrhage events and compared between each other using the paired t-test. Receiver operating characteristic (ROC) curves were plotted for the most robust ratios. Cut-off values for weak, moderate and strong indicators of severe haemorrhage were obtained by examining the ROC curve; whereby, mild haemorrhage events had to have a 100% specificity (indicating the cat has not undergone severe haemorrhage) and severe haemorrhage events had to have 100% sensitivity (indicating the cat has undergone severe haemorrhage). The sensitivity and specificity values were calculated for weak, moderate and strong indicators of severe haemorrhage. Weak values required to be 100% specific and the highest value for sensitivity at the interval; and strong values had to be 100% sensitive and the highest value for specificity at the interval. Moderate values were in between the weak and strong values. The positive and negative likelihood ratios and predictive values for each cut-off value of the indicators were then estimated. The predictive values were calculated using a prevalence of 50% for severe haemorrhage.

Data were analysed using commercially available software (MiniTab 18.1; Minitab Inc., PA, USA; and MedCalc 19.0.3; MedCalc Software; Belgium) and significance interpreted at $p < 0.05$. Data were reported as mean (± standard deviation).

2.4 Results

All cats completed both haemorrhage events. The FE´Iso was 1.7% ± 0.2% and 1.8% ± 0.1% during the mild and severe haemorrhage events, respectively (t-value: -1.37; $p = 0.229$). Blood loss was 4.5 ± 1.1 mL kg^{-1} for the mild haemorrhage event and 26.8 ± 5.5 mL kg^{-1} for the severe haemorrhage event. Haemorrhage time was 15.3 ± 0.4 minutes for the mild haemorrhage event and was not different from 17.2 ± 2.6 minutes for the severe haemorrhage event (t-value: -1.53; $p = 0.186$). The MAP after the mild haemorrhage event was 64 ± 4 mmHg and significantly greater than 44 ± 4 mmHg following the severe haemorrhage event (t-value: 10.49; $p < 0.01$). These outcomes indicate that all cats were subjected to haemorrhage events that met the study requirements. The outcomes of the initial screening analyses of the 34 variables are presented in **Table 2.1**.

Table 2.1 Difference and percent change in physiological, haematological, biochemical, electrolyte, blood gas and acid-base variables pre- and post-haemorrhage in anaesthetised cats undergoing mild and severe haemorrhage events. The variables were ordered from largest to smallest size effect (Cohen d value) and only values > 0.8 were considered as large and clinically meaningful.

Parameter	Value type	Mild Haemorrhage		Severe Haemorrhage		Paired t-test		
		Mean	SD	Mean	SD	95%CI - μ_d	Cohen *d*	(t-value; *p*-value)
				Physiological				
SAP	Difference	-1	4	-23	9	(14; 31)	3.7	(7.1; <0.01)
(mmHg)	% Change	-0.6	4.6	-26.5	9.4	(16.6; 35.3)	3.8	(7.13; <0.01)
MAP	Difference	-5	8	-27	6	(18; 27)	3.4	(13.44; <0.01)
(mmHg)	% Change	-5.9	11.4	-38.0	5.2	(24; 40)	4.0	(10.38; <0.01)
Heart rate	Difference	3	8	49	23	(-66; -26)	-2.9	(-5.97; <0.01)
(beats min^{-1})	% Change	1.9	6.7	44.5	21.0	(-62.8; -22.4)	-3.0	(-5.42; <0.01)
DAP	Difference	-5	8	-21	6	(11; 20)	2.5	(8.69; <0.01)
(mmHg)	% Change	-7.9	12.4	-35.9	6.5	(19.3; 36.6)	3.1	(8.29; <0.01)
$PE'CO_2$	Difference	0	2	-5	4	(2; 9)	1.2	(3.57; 0.02)
(mmHg)	% Change	0.2	5.5	-11.0	9.9	(5.2; 17.3)	1.5	(4.77; <0.01)
Vtexp	Difference	1	1	0	3	(-3; 4)	0.4	(0.47; 0.65)
(mL)	% Change	3.9	4.9	2.5	13.2	(-16.5; 19.4)	0.2	(0.21; 0.84)
f_R	Difference	0	5	1	6	(-4; 2)	-0.2	(-1.05; 0.34)
(breaths min^{-1})	% Change	-3.8	14.8	18.2	29.0	(-42.7; -1.4)	-1.0	(-2.74; 0.04)
Temperature	Difference	-0.1	0.2	-0.1	0.4	(-0.42; 0.32)	-0.2	(-0.35; 0.74)
(°C)	% Change	-0.4	0.4	-0.2	1.2	(-1.2; 0.9)	-0.2	(-0.34; 0.75)
SpO_2	Difference	0	1	1	1	(-1.4; 1.1)	-0.2	(-0.35; 0.74)
(%)	% Change	0.0	1,1	0.2	0.4	(-1.4; 1.1)	-0.2	(-0.34; 0.75)
				Haematology				
Haemoglobin	Difference	-3	2	10	10	(-24; -1)	-1.8	(-2.73; 0.04)
(mg dL^{-1})	% Change	-3.2	2.5	11.2	12.5	(-28.2; -0.5)	-1.7	(-2.67; 0.04)
Erythrocyte count	Difference	5.9	0.7	6.8	1.0	(-1.6; -0.3)	-1.5	(-3.51; 0.02)
	% Change	-1.7	2.3	9.9	12.9	(-26.9; 3.6)	-1.4	(-1.96; 0.12)
Haematocrit	Difference	0.00	0.01	0.03	0.03	(-0.07; 0.01)	-1.4	(-2.19; 0.08)
(L/L)	% Change	-1.8	3.1	10.4	13.1	(-26.8; 2.5)	-1.4	(-2.13; 0.09)
Leukocyte count	Difference	-0.4	0.5	-1.6	1.3	(0.1; 2.3)	1.3	(2.78; 0.04)
	% Change	-3.8	3.4	-18.8	11.6	(2.6; 27.5)	1.9	(3.11; 0.03)
Thrombocyte count	Difference	4.8	44	-38	59	(-32; 116)	0.9	(1.46; 0.20)
	% Change	1.8	15.5	-11.1	18.7	(-11.1; 36.9)	0.8	(1.38; 0.23)
Neutrophil count	Difference	-0.04	0.28	-0.41	1.01	(-0.83; 1.58)	0.6	(0.80; 0.46)
	% Change	0.0	5.6	-9.5	15.2	(-9.0; 27.8)	0.9	(1.31; 0.25)
Lymphocyte count	Difference	-0.3	0.5	-0.4	1.3	(-0.9; 1.1)	0.1	(0.32; 0.76)
	% Change	-7.5	13.2	1.8	41.2	(-52.5; 33.8)	-0.3	(-0.56; 0.60)

Table 2.1 continued								
Biochemistry								
Albumin	Difference	-1	0	-4	2	(1; 5)	2.3	(4.4; <0.01)
(g L^{-1})	% Change	-2.5	2.0	-12.1	6.2	(4.3; 15.0)	2.3	(4.66; <0.01)
Creatinine	Difference	-1	3	13	10	(-25; -3)	-2.1	(-3.32; 0.02)
(mmol L^{-1})	% Change	-0.8	2.1	12.6	9.2	(-23.1; -3.7)	-2.2	(-3.55; 0.02)
Glucose	Difference	0.0	0.2	0.8	1.0	(-1.9; 0.17)	-1.3	(-2.16; 0.08)
(mmol L^{-1})	% Change	-1.2	3.8	14.9	16.5	(-32.7; 0.5)	-1.5	(-2.49; 0.06)
BUN	Difference	0	0	1	0	(-0.9; 0.1)	-1.3	(-1.87; 0.12)
(mmol L^{-1})	% Change	1.1	1.8	5.8	5.1	(-10.9; 1.6)	-1.3	(-1.90; 0.12)
Lactate	Difference	0.0	0.3	0.0	0.5	(-0.6; 0.6)	<0.1	(-0.07; 0.95)
(mmol L^{-1})	% Change	3.9	20.6	3.2	30.4	(-40.8; 42.3)	<0.1	(0.04; 0.97)
Electrolytes								
Potassium	Difference	0.1	0.1	0.3	0.2	(-0.4; -0.1)	-1.5	(-3.53; 0.02)
(mmol L^{-1})	% Change	2.8	2.5	9.6	6.4	(-11.9; -1.7)	-1.5	(-3.41; 0.02)
Magnesium	Difference	0.8	0.1	0.8	0.1	(-0.03; 0.09)	1.0	(1.31; 0.25)
(mmol L^{-1})	% Change	0.3	3.5	-2.2	2.0	(-0.3; 5.2)	0.9	(2.33; 0.07)
Phosphorus	Difference	0	0	0	0	(-0.3; 0.1)	-0.8	(-1.05; 0.34)
(mmol L^{-1})	% Change	2.5	2.9	6.1	6.9	(-13.0; 5.8)	-0.8	(-0.99; 0.37)
Chloride	Difference	-1	4	1	2	(-5; 2)	-0.6	(-1.36; 0.23)
(mmol L^{-1})	% Change	-0.9	3.3	0.6	2.0	(-4.5; 1.5)	-0.6	(-1.28; 0.26)
Sodium	Difference	-2	6	0	6	(-8; 5)	-0.3	(-0.65; 0.54)
(mmol L^{-1})	% Change	-1.3	3.9	-0.3	4.4	(-5.7; 3.6)	-0.3	(-0.58; 0.59)
Ionised calcium	Difference	0	0.1	0	0.1	(-0.1; 0.1)	-0.1	(-0.17; 0.87)
(mmol L^{-1})	% Change	0.7	6.2	1.0	8.8	(-11.2; 10.5)	-0.1	(-0.09; 0.93)
Blood gases and acid-base balance								
Bicarb (act)	Difference	0.1	0.4	-1.5	0.8	(0.9; 2.2)	2.7	(6.01; <0.01)
(mmol L^{-1})	% Change	0.3	2.0	-7.8	4.2	(4.8; 11.4)	2.7	(6.30; <0.01)
PaO_2	Difference	-14	23	53	32	(-91; -43)	-2.6	(-7.00; <0.01)
(mmHg)	% Change	-4.0	6.3	14.4	9.2	(-25.2; -11.6)	-2.6	(-6.93; <0.01)
$PaCO_2$	Difference	0	1	-5	3	(3; 8)	2.2	(5.01; <0.01)
(mmHg)	% Change	0.4	4.4	-12.3	8.6	(6.9; 18.5)	2.0	(5.61; <0.01)
Base Excess	Difference	0.1	0.4	-1.1	1.1	(0.1; 2.2)	1.6	(2.84; 0.04)
(mmol L^{-1})	% Change	-1.2	6.7	22.9	36.7	(-1.2; 22.9)	-1.0	(-1.68; 0.15)
$PvCO_2$	Difference	-2	4	-4	2	(-2; 6)	0.8	(1.44; 0.21)
(mmHg)	% Change	-3	7	-7	5	(-3.4; 12.1)	0.9	(1.61; 0.17)
pH	Difference	0.00	0.02	0.02	0.04	(-0.05; 0.01)	-0.8	(-1.58; 0.18)
	% Change	0.0	0.2	0.3	0.5	(-0.7; 0.2)	-0.8	(-1.60; 0.17)
Bicarb (std)	Difference	-1.7	3.8	-0.7	0.9	(-5.4; 3.4)	-0.4	(-0.59; 0.58)
(mmol L^{-1})	% Change	-8.6	19.7	-3.2	4.1	(-27.7; 17.1)	-0.4	(-0.61; 0.57)

SD: standard deviation; 95% CI - μ_d: 95% confidence interval of the difference between the means; SAP: systolic arterial blood pressure; MAP: mean arterial blood pressure; DAP: diastolic arterial blood pressure; $PE'CO_2$: end-tidal carbon dioxide; Vtexp: expiratory tidal volume; f_R: respiratory rate; SpO_2: peripheral oxygen haemoglobin saturation; BUN: blood urea nitrogen; Bicarb (act): actual bicarbonate ion concentration; PaO_2: arterial partial pressure of oxygen; $PaCO_2$: arterial partial pressure of carbon dioxide; $PvCO_2$: venous partial pressure of carbon dioxide; Bicarb (std): standard bicarbonate ion concentration.

The qualifying screening physiological variables were the HR (*d* = -2.9), arterial blood pressure (SAP [*d* = 3.7], MAP [*d* = 3.4] and DAP [*d* = 2.5]) and $PE'CO_2$ (*d* = 1.7). Out of the haematological variables, haemoglobin concentration (*d* = -1.8) and leukocyte count (*d* = 1.3) qualified as potential screening variables. The Ht (*d* = -1.4) was also included in the ratio analysis to determine its suitability because its trend in difference and percent change were similar to Hb. Out of the biochemical variables, serum albumin (*d* = 2.3) demonstrated a more robust difference and percent change compared to creatinine (*d* = -2.1) and glucose (*d* = -1.3), both of which were also screening criteria fits. Potassium (*d* = -1.5) was the only electrolyte that demonstrated a difference and percent change. For the blood gases variables, $PaCO_2$ (*d* = 2.2) and PaO_2 (*d* = -2.6) and actual bicarbonate ion concentration (*d* = 2.7) qualified as screening variables. The $PaCO_2$ tended to shift in similar direction to $PE'CO_2$.

The 15 variables that met the initial screening criteria qualifications were placed into 13 different ratios for further analysis (not all ratios are reported due to either a lack of differentiation between haemorrhage states or for being mathematically-difficult to interpret values) and are presented in **Table 2.2**. The most robust ratios, demonstrating clinically relevant sensitivity and specificity within each indicator cut-off range, where the 1) shock index (*d* = -2.8; HR:SAP), 2) HR:$PE'CO_2$ (*d* = -2.9), 3) serum albumin:Ht (*d* = 1.5), and 4) HR: actual bicarbonate ion concentration (*d* = -1.6).

Table 2.2 Comparison of screening qualifying ratios using the paired t-test and Cohen's d in anaesthetised cats undergoing mild and severe haemorrhage events.

Ratio	Mild Haemorrhage		Severe Haemorrhage		Paired t-test		
	Mean	SD	Mean	SD	95%CI - μ_d	Cohen *d*	(t-value; *p*-value)
HR:$PE'CO_2$	2.5	0.5	4.2	0.7	(-2.3; -1.2)	-2.9	(-8.53; <0.01)
HR:SAP (SI)	1.3	0.3	2.5	0.6	(-1.7; -0.8)	-2.8	(-7.44; <0.01)
HR:$PaCO_2$	2.6	0.6	4.2	1.1	(-2.9; -0.3)	-2.0	(-3.45; 0.03)
HCO_3(act):Ht	71	7	57	10	(5; 22)	1.8	(3.91; 0.01)
HR:HCO_3(act)	6	2	10	3	(-5; -2)	-1.6	(-5.97; <0.01)
Alb:Ht	108	15	85	18	(11; 35)	1.5	(4.82; <0.01)
Alb:Hb	0.34	0.05	0.26	0.06	(0.03; 0.11)	1.5	(4.30; <0.01)

SD: standard deviation; 95% CI - μ_d: 95% confidence interval of the difference between the means; HR: $PE'CO_2$: heart rate to end-tidal carbon dioxide; SI: shock index calculated by dividing the heart rate by the systolic arterial blood pressure; HR: $PaCO_2$: heart rate to arterial partial pressure of carbon dioxide; HCO_3(act):Ht: actual bicarbonate ion concentration to haematocrit; HR:HCO_3(act): heart rate to actual bicarbonate ion concentration; Alb:Ht: serum albumin concentration to haematocrit; Alb:Hb: serum albumin concentration to haemoglobin concentration.

The cut-off values for weak, moderate and strong indicators of severe haemorrhage determined from the ROC curves yielded clinically meaningful sensitivities and specificities, corroborated by meaningful likelihood ratios and predictive values; and are presented in **Table 2.3** and **Figure 2.1**.

Table 2.3 Sensitivity, specificity and positive and negative likelihood ratios and predicative values of three ratios that make up an acute haemorrhage scoring system in anaesthetised cats. The cut-off values divide the ratio value into weak, moderate and strong indicator strengths whereby a cat obtaining values within the strong indicator would have most likely sustained a severe haemorrhage in excess of 22 mL kg^{-1}.

Ratio	Indicator	Value	Sensitivity	Specificity	+LR	-LR	+PV	-PV	AUC	Z-statistic
HR:SAP (SI)	Weak	<1.3	83	100	6	0.17	100	86	0.986	24.7
	Moderate	1.4 to 1.9	100	83	6	0.01	86	100		
	Strong	>2.0	100	67	3	0.01	75	100		
Alb:Ht	Weak	>120	17	100	2	0.83	100	55	0.833	2.6
	Moderate	91 to 119	67	83	4	0.40	80	71		
	Strong	<90	100	67	3	0.01	75	100		
HR:PE′CO_2	Weak	<2.5	60	100	6	0.40	100	75	>0.999	-
	Moderate	2.6 to 3.6	100	83	6	0.01	83	100		
	Strong	>3.7	100	67	3	0.01	71	100		
HR:HCO_3(act)	Weak	< 6.5	83	100	5	0.17	100	86	0.917	4.6
	Moderate	6.6 to 9.8	83	83	5	0.20	83	83		
	Strong	> 9.9	100	50	2	0.01	67	100		

+LR: positive likelihood ratio; -LR: negative likelihood ratio; +PV: positive predictive value; -PV: negative predictive value; AUC: area under the curve; SI: shock index calculated by dividing the heart rate by the systolic arterial blood pressure; Alb:Ht: serum albumin concentration to haematocrit; HR: PE′CO_2: heart rate to end-tidal carbon dioxide; HR:HCO_3(act): heart rate to actual bicarbonate ion concentration.

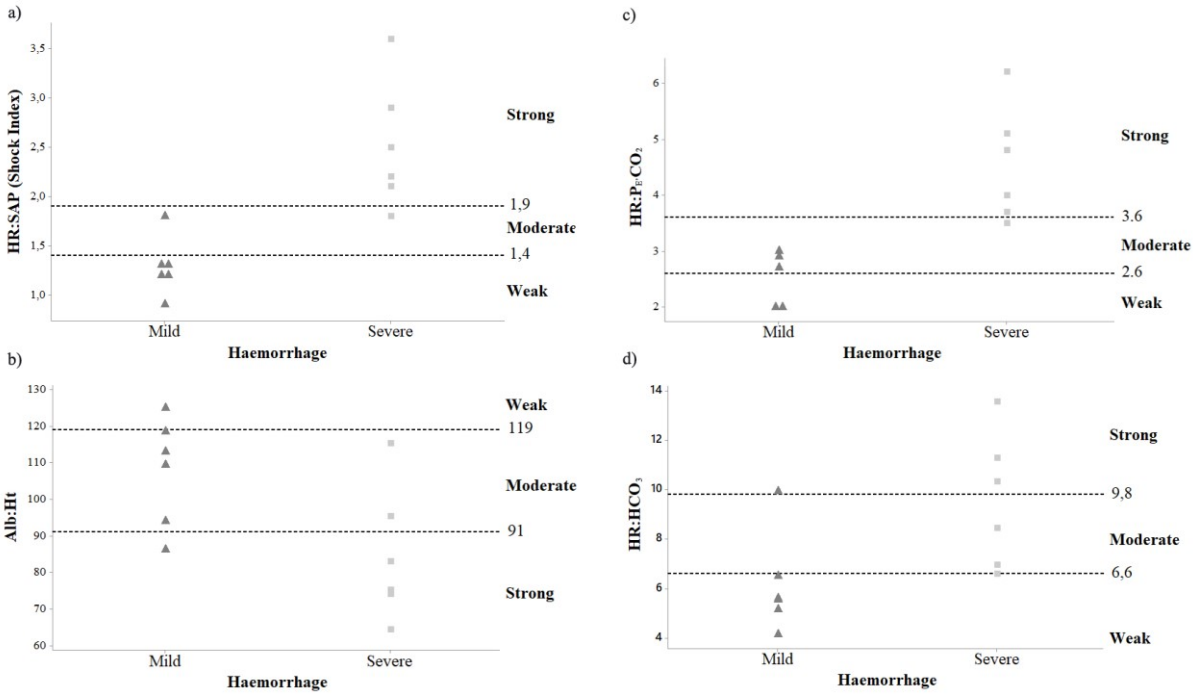


Figure 2.1 Individual value plots of a) shock index, b) serum albumin: haematocrit, c) heart rate: end-tidal carbon dioxide, and d) heart rate: actual bicarbonate ion concentration ratios in cats that underwent a mild and severe haemorrhage event while under general anaesthesia. The horizontal reference lines indicate the cut-off values among the indicators (weak, moderate, strong) of severe haemorrhage. Values within the strong area of the plot indicate that a severe haemorrhage has likely occurred. Where: HR:SAP: shock index calculated by dividing the heart rate by the systolic arterial blood pressure; Alb:Ht: serum albumen concentration to haematocrit; HR:PE′CO_2: heart rate to end-tidal carbon dioxide; HR:HCO_3: heart rate to actual bicarbonate ion concentration.

These four ratios were included into the final proposed CABSS (**Table 2.4**). We suggest that the CABSS would apply to cats that are experiencing ongoing haemorrhage or up to 15 minutes after acute haemorrhage.

Table 2.4 Cat Acute Bleeding Scoring System (CABSS) which is an intraoperative haemorrhage scoring system proposed to detect and quantify severe haemorrhage in anaesthetised adult cats.

Ratio	Direction of change over time†	Indicator strength		
		Weak	**Moderate**	**Strong**
Must calculate these two ratios as minimum				
HR:SAP (SI)	Increasing	**< 1.3**	**1.4 to 1.9**	**> 2.0**
HR:PE′CO_2	Increasing	**< 2.5**	**2.6 to 3.6**	**> 3.7**
Assess one or both ratios below to increase suspicion of severe haemorrhage				
Alb:Ht	Decreasing	**> 120**	**91 to 119**	**< 90**
HR:HCO_3(act)	Increasing	**< 6.5**	**6.6 to 9.8**	**< 9.9**
Interpretation of ratios				
Haemorrhage		***Mild***		***Severe***
Blood loss (mL kg^{-1})		***< 10***	***11 to 21***	***> 22***
User instructions: All ratios should be calculated to accurately detect and quantify blood loss during acute intraoperative haemorrhage. In the event that not all variables can be measured then, at a minimum, the HR:SAP and HR:PE′CO_2 should be calculated. Footnote: †Change in direction of the variable is compared to preoperative values.				

HR: heart rate; SAP: systolic arterial blood pressure (mmHg); SI: shock index; PE′CO_2: end-tidal carbon dioxide (mmHg); Alb: serum albumin concentration (g L^{-1}); Ht: haematocrit (L/L^{-1})

The serum albumin:Ht could be substituted by the serum albumin:Hb (*d* = 1.5), however, the ratio values obtained are mathematically difficult to interpret because of the high number of decimal places and cannot be recommended (**Table 2.2**). The HR:PE′CO_2 could be substituted by the HR:$PaCO_2$ (*d* = -2.0) and conform to the suggested indicator cut-off ranges for this ratio. However, the HR:$PvCO_2$ cannot be recommended as a substitute for the HR:PE′CO_2 ratio because it did not pass the initial screening criteria.

2.5 Discussion

Anaesthetised cats that have undergone mild and severe haemorrhage demonstrated reliable differences in various pre- and post-haemorrhage variables assessed in the present study. The most notably different variables were HR, SAP, PE′CO_2, serum albumin, Ht, and actual bicarbonate ion concentration. Four easy to calculate ratios were derived from these variables and included 1) shock index, 2) serum albumin:Ht, 3) HR:PE′CO_2, and 4) HR: actual bicarbonate ion concentration. These ratios make up the proposed Cat Acute Bleeding Scoring System (CABSS) which aims to quantify

haemorrhage severity by indicating (weak, moderate and strong) the likelihood of severe haemorrhage in cats.

The shock index which was first described in 1967 is perhaps the most commonly investigated human scoring system that has been studied in dogs (Allgower & Burri 1967). A shock index of > 1.0 is considered a sensitive (85%) and specific (80%) tool used to detect small volume blood loss (≈18% blood loss) in healthy dogs donating blood, and more useful compared to monitoring the haematocrit, lactate and total plasma proteins (McGowan et al. 2017). Furthermore, the shock index was also a useful triage tool in dogs presenting with haemorrhagic shock, and values > 0.9 indicate that further investigation to rule out severe and ongoing haemorrhage should be done to stabilise the patient (Peterson et al. 2013). The shock index only makes use of the classic cardiovascular variables that change over time in response to hypovolaemia where the HR increases to compensate for the decrease in blood pressure. However, human anaesthesiologists Schultz and McConachie (2015) caution the use of vital signs alone during haemorrhage because there are patients that develop uncommon neurovascular reflexes that alter the classic physiological response to hypovolaemia, such that they may present with seeming normal vital signs. Furthermore, cats that were administered medetomidine as part of a general anaesthetic protocol have a shock index of < 1.0 (Zeiler et al. 2014). However, the cardiovascular effects of medetomidine, or other drugs used during the perianaesthetic period might mask the classic cardiovascular response to hypovolaemia and this masking could confound intraoperative scoring during severe haemorrhage. Also, cats can present with a normal HR or develop bradycardia during shock and therefore the shock index could be < 1.0 (Murphy & Hibbert 2013). To avoid misdiagnosis, we have incorporated other ratios into our proposed scoring system.

The HR:PE′CO_2 ratio demonstrated an increasing trend whereby the HR increased and the PE′CO_2 decreased as the volume of blood loss increased. The decreasing PE′CO_2 and $PaCO_2$, and the increase in HR suggest a decrease in cardiac output (Dubin et al. 1990; Long et al. 2017). Furthermore, Dubin et al. (1990) found that the $PvCO_2$ increases during low cardiac output states like hypovolaemia, widening the gap between the $PaCO_2$ and $PvCO_2$. Our data agree with the findings reported by Dubin et al. (1990). Venous hypercarbia and arterial hypocarbia have been described in dogs and rabbits during low flow states as a result of haemorrhagic shock (Benjamin et al. 1987; Williams et al. 2014).

This effect of venous hypercarbia during low flow states confirms our finding that the $PvCO_2$ cannot be used as a substitute for the $PE'CO_2$.

The serum albumin:Ht ratio reflected acute changes within the intravascular compartment during haemorrhage. The Ht increased and serum albumin concentration decreased, therefore the serum albumin:Ht ratio demonstrated a consistent decrease. The rise in Ht in the cats subjected to severe haemorrhage has not been consistently described in other experimental models, furthermore decrease has been described (Groom et al. 1965; Mott 1967). This inconsistency could be related to the rate of haemorrhage and time of blood sampling to test the Ht. Our cats were bled over 15 minutes and sampled immediately after haemorrhage, whereas their measurements were over hours, allowing their cats enough time for fluid shifts to take place (Jutkowitz 2004). However, in dogs, a rise in Ht occurs after acute haemorrhage and before fluid shifts take place or before fluid resuscitation (Kirby 1995; McGowan et al. 2017). The decrease in albumin concentration could have been because of the loss of whole blood (Kirby 1995).

The HR: actual bicarbonate ion concentration ratio also reflected acute changes within the intravascular compartment during haemorrhage. The actual bicarbonate ion concentration is scantly reported in cats, especially for those in haemorrhagic shock. Regardless, the actual bicarbonate ion is a value that is calculated by modern blood gas analysing machines using the Henderson-Hasselbalch equation of pH and reflects the entire concentration of bicarbonate ions within the blood (Lawrrie & Golda 1979). This ratio performed well and warrants further investigation, and was thus incorporated into the CABSS. The CABSS could be a useful tool in diagnosing and quantifying haemorrhage volumes in cats undergoing surgical procedures under general anaesthesia. Furthermore, the CABSS could be a useful tool to gauge haemorrhage volumes in traumatised cats that present to veterinary hospitals within 30 minutes of sustaining trauma.

The present study has two notable limitations. The sample size is small so only had the power to reliably to detect only large effects; and even then, the magnitude of the effect sizes is likely to be biased. Nevertheless, the variables included in the ratios are physiologically justified, and they yielded robust thresholds for differentiating mild and moderate haemorrhage. Furthermore, the observations herein will assist future researchers in estimating adequate samples sizes to validate this scoring system under various clinical conditions. The second limitation is that the study was conducted in

anaesthetised cats under controlled conditions. Therefore, extrapolation of our findings to trauma or conscious animals or those given different drugs during anaesthesia will require further investigation. However, the authors speculate that the variables that make up the CABSS ratios should result in a similar outcome regardless of the clinical presentation. Our assumption is based on us using an anaesthetic protocol that had minimal effects on the cardiovascular system. Furthermore, we did not titrate the isoflurane concentration (1 x minimum alveolar concentration [1 MAC] recommended for surgery in cats; Shaughnessy & Hofmeister 2014) to effect.

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2.6 Conclusions

Cats subjected to mild and severe haemorrhage demonstrated statistically and clinically relevant change in HR, SAP, $PE'CO_2$, serum albumin, Ht and actual bicarbonate ion concentration ratios. The four ratios that were created from these variables and the unitless value of these ratios, when assessed together, aided the detection and quantification of severe haemorrhage in this study. The four ratios make up the proposed Cat Acute Bleeding Scoring System (CABSS) which could be a tool for detecting and quantifying acute haemorrhage in cats.

CHAPTER 3

Biomarkers that indicate an endpoint for administering lactated Ringer's solution or 6% tetrastarch 130/0.4 fluids to resuscitate haemorrhage-induced hypovolaemic anaesthetised cats: a concept of volume-endpoints to prevent iatrogenic fluid overload

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3.1 Abstract

Objectives To determine volume-endpoint biomarkers for intravenous fluid administration in haemorrhage-induced hypovolaemic hypotensive anaesthetised cats.

Study design Randomised crossover study.

Animals Six domestic cats (21 ± 1 months old; 4.9 ± 1.2 kg).

Methods The cats underwent three treatments, 2 months apart. They were anaesthetised (buprenorphine, alfaxalone, isoflurane in oxygen, fixed end-tidal concentration of 1.7%) and instrumented to measure a wide range of physiological, blood gas, haematological and biochemical variables over time. Samples were taken during a health check, before haemorrhage, after haemorrhage and then at 30-minute intervals during fluid resuscitation and the next day. The three treatments were: 1) control, mild haemorrhage and sham resuscitation; 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) where the cats underwent a controlled haemorrhage followed by resuscitation where they were administered LRS and Vol at 60- and 20-ml kg^{-1} $hour^{-1}$, respectively for 120 minutes. Fluid overload was identified by oedema, nasal discharge and radiographic evidence of pleural oedema or effusion. Data over time were analysed using a general linear mixed model and significant variables were then analysed using receiver operating characteristic curves.

Results The total blood loss was 10.2 ±2.3, 29.3 ±9.0 and 29.1 ±6.3 mL kg-1 for control, LRS and Vol, respectively. The total volume of LRS and Vol administered was 120 and 40 mL kg-1, respectively. Overall, haematocrit, serum albumin, total magnesium, chloride to sodium ratio, sodium chloride difference demonstrated potential variables that could determine a volume-endpoint. These variables shifted in value out of normal clinical ranges for cats from 30 minutes of resuscitation onwards. A chloride to sodium ratio > 0.84 is sensitive (90%) and specific (75%) as a volume-endpoint biomarker.

Conclusions and relevance Volume-endpoints for haemorrhage-induced hypovolaemic hypotension in anaesthetised cats were identified. Overall, physiological variables, haematocrit and serum albumin concentration are not good indicators of circulating volume restoration compared to chloride to sodium ratio and magnesium concentration. Finding the ideal biomarker to identify adequate volume resuscitation of commonly used intravenous fluids will improve the overall safety of their administration and prevent fluid overload in cats.

3.2 Introduction

Fluid overload occurs in veterinary medicine, however, most of the information is anecdotal or based on retrospective studies (Cavanagh et al. 2016; Thomovsky et al. 2016; Ostroski et al. 2017). There is no definition of fluid overload in human or veterinary medicine, but it occurs in nondehydrated animals when the amount of fluid entering exceeds the amount of fluid lost from the body (Thomovsky et al. 2016). An increase in body weight by more than 10% from baseline because of fluid administration could result in the clinical manifestation of fluid overload (Thomovsky et al. 2016). Early detection of fluid overload is difficult because of similar physiological responses during anaesthesia, disease or trauma that mask its subtle manifestation. The discrete clinical signs include peripheral oedema formation, cavity effusions and fluid drainage from surgical sites and respiratory airways. Diagnostic imaging (specifically radiographs and ultrasound) can aid in confirming fluid overload (Thomovsky et al. 2016). When these discrete clinical signs occur then, unfortunately, there are few interventions that can relieve fluid overload. The sequelae of fluid overload are associated with organ oedema and effusions, which generally culminate in a poor outcome because of poor microvascular tissue perfusion and oxygenation (Malbrain et al. 2018). In cats, reports of fluid overload are scant, but when it occurs it is associated with increased cost of treatment and length of hospitalisation, but not with an increased mortality (Ostroski et al. 2017). Fluid therapy in cats has been associated with increased odds of death during anaesthesia and sedation (Brodbelt et al. 2007).

Several comparable best-evidence fluid therapy plans have been conceptualised, but ROSE (Resuscitation, Optimisation, Stabilisation, and Evacuation) has been frequently advocated (Thomovsky et al. 2016; Malbrain et al. 2018). The clinical manifestation of fluid overload lags behind the fluids being administered. We speculate that fluid overload occurs during the resuscitation and optimisation phases of fluid therapy. During the resuscitation phase, the patient is fluid-dependent and must receive ‘enough’ fluids, to correct the volume deficit, with the goal of improving cardiac output and oxygen delivery as quickly as possible (Britt et al. 1996; Davis et al. 2013). The optimisation phase is when fluids have been administered for resuscitation, but hypotension, low cardiac output and low oxygen delivery persist. Therefore, administration of more fluids, or a mixture of different types of fluids are required to restore the intravascular volume and function to normal. Cardiovascular and oxygenation

biomarkers are used to monitor resuscitation and have been used to indicate the end of the resuscitation and optimisation phases of fluid therapy and are used to determine the animal's response to fluids.

When these biomarkers are used to determine the response to fluid administration, they are intended to identify fluid responders (measured biomarkers improve when fluids are administered). However, there are no clear guidelines on how much fluid should be administered before it is decided that the patient is a fluid non-responder (Prittie 2006). We speculate that fluid non-responders are prone to developing fluid overload. Furthermore, individualised physiological responses to disease processes, trauma and acute haemorrhage prevent the reliable identification of fluid responders. Therefore, instead of investigating biomarkers for resuscitation-endpoints or fluid responders, it would be clinically relevant to identify volume-endpoint biomarkers that can guide fluid administration. Specifically, volume-endpoints should indicate a point where enough fluid has been administered, independent of the response to resuscitation. If there is an inadequate response to the fluid therapy during the resuscitation and optimisation phases when a volume-endpoint is reached, then the overall resuscitation plan should be revised to include other therapies like catecholamine infusions to achieve the cardiovascular and oxygenation goals of the resuscitation. Therefore, proposed volume-endpoints should not be confused with resuscitation-endpoints. The volume-endpoints could prompt early resuscitation plan revision and avoid fluid overload more reliably compared to current fluid therapy practices.

During haemorrhage-induced hypovolemic hypotension, it is critical to restore the intravascular volume (volume resuscitation) rapidly to avoid haemorrhagic shock. In veterinary medicine, during fluid therapy to treat controlled severe haemorrhage (> 40% of blood volume lost), fluids are administered either at a shock dose rate or at a ratio dose. In cats, the shock dose rate for isotonic crystalloid fluids is 40-60 ml kg^{-1} $hour^{-1}$ and 5-15 ml kg^{-1} $hour^{-1}$ for synthetic colloid fluids (Rozanski & Rondeau 2002). If the volume of blood lost is known, or estimated, then these fluids can be administered at various ratios to the lost blood. Isotonic crystalloid fluids are administered at a ratio of 3:1 (3 ml of fluid administered per 1 ml of whole blood loss). The administration ratio for synthetic colloids is 1:1 (Broadstone 1999). These doses have not been widely investigated in cats and their fluid tolerance is not well understood.

Investigators are required to collect as much data as possible using different modalities to determine trends over time within a variable in animals undergoing haemorrhage and resuscitation to determine effective biomarkers (Marshall & Reinhart 2009). Currently, there is very little information on fluid

resuscitation in cats and there is a general assumption that this species is prone to fluid overload, especially in situations of severe haemorrhage (Rudloff & Kirby 1994). We aimed to determine unique biomarkers that can be used as volume-endpoints of fluid administration during treatment of severe acute haemorrhage using an isotonic crystalloid or isooncotic colloid fluid.

3.3 Materials and Methods

3.3.1 Animals

A group of six neutered domestic cats (three male and three female; aged 21 ± 1-months; 4.9 ± 1.2 kg) were used in the study. They were housed in a communal indoor-outdoor cattery. The animal ethics committees of the University of Pretoria (v006-15) and University of Witwatersrand (2017-10-68-C-AREC) approved the study.

The cats underwent a health status check that included a physical examination and venous blood analyses one week before the treatments. Food, but not water, was withheld eight hours before treatment.

3.3.2 Study procedures

Cats were randomly assigned (balanced single block design; www.randomization.com) to receive three treatments each at 2-month intervals. On the morning of treatment, the cat underwent a clinical examination, was weighed and premedicated with buprenorphine hydrochloride (0.02 mg kg^{-1}; Temgesic 0.3 mg/ml; Reckitt Benckiser Healthcare, UK) intramuscularly and left alone for 45 minutes. An indwelling catheter (22 gauge; Jelco; Smith Medical International, UK) was aseptically inserted into one of the cephalic veins and secured. Alfaxalone (Alfaxan-CD RTU 10mg ml^{-1}; Afrivet, RSA), titrated to induce general anaesthesia, was administered intravenously. The trachea was intubated with a cuffed endotracheal tube, which was secured in place and connected to a paediatric circle breathing

system (15 mm internal diameter). General anaesthesia was maintained with isoflurane (Isofor; Safeline Pharmaceuticals, RSA), initially set to 2%, in oxygen at a fixed flow rate of 80 ml kg^{-1} $minute^{-1}$. A standardised target end-tidal isoflurane concentration for the study was set at 1.7% (Shaughnessy & Hofmeister 2014). The ventral neck region was clipped and surgically scrubbed before the cat was transferred to the procedure table. An isotonic crystalloid fluid (Lactated Ringer's solution; Fresenius Kabi, RSA) was infused, for maintenance, by an electronic device at 5 ml kg^{-1} $hour^{-1}$ via a y-ported administration set connected to the cephalic catheter. The thorax was radiographed by taking two orthogonal views (dorso-ventral and right lateral). The cat was then placed in dorsal recumbency and a final surgical preparation of the ventral neck was done. Probes and leads were placed and connected to a multiparameter machine (Datex-Ohmeda Cardiocap 5; Datex; Finland) to monitor physiological variables as follows: heart rate by electrocardiogram, peripheral oxygen haemoglobin saturation by pulse oximetry, end-tidal carbon dioxide and respiratory rate by capnometry, and oesophageal temperature. The end-tidal isoflurane concentration was monitored and the vaporiser adjusted to maintain a standardised concentration of 1.7%. A catheter (22 Gauge, 50 mm, Arrow arterial catheterization set; Arrow International; PA, USA) was inserted into the right pre-superficialised (superficialised 15 months earlier during gonadectomy) carotid artery by cut-down technique to allow for invasive blood pressure monitoring and intermittent blood sampling for gas tension analyses. A catheter (22 Gauge, 50 mm, Arrow arterial catheterization set; Arrow International; PA, USA) was inserted percutaneously into the left jugular vein for intermittent blood sampling and facilitation of controlled haemorrhage later. Both catheters were inserted using the Seldinger technique (Seldinger 1953). One of the three randomly allocated treatments was then commenced. The treatments were divided into two phases, the haemorrhage phase and resuscitation phase, as follows:

Control: A waiting period of 15 minutes duration to simulate controlled haemorrhage followed by sham resuscitation of 120 minutes duration.

Lactate Ringer's solution (LRS): controlled haemorrhage followed by LRS administration at 60 ml kg^{-1} $hour^{-1}$ for 120 minutes during resuscitation.

6% Tetrastarch 130/0.4 (Vol): controlled haemorrhage followed by Vol (Voluven 130/0.4; Fresenius Kabi) administration at 20 ml kg^{-1} $hour^{-1}$ for 120 minutes during resuscitation.

During the controlled haemorrhage phase blood was withdrawn manually into a semi-closed system using 20 ml syringes primed with citrate-phosphate-dextrose (4 ml; JMS blood bag, 450 ml; JMS Singapore) via the jugular catheter at a targeted rate of 2 ml kg^{-1} $minute^{-1}$ (target of 15 minutes) until one of two endpoints: 1) maximum blood withdrawal of 30 ml kg^{-1}; or 2) mean arterial blood pressure of < 48 mmHg that persisted for at least 3 minutes.

During the resuscitation phase the randomised resuscitation fluid was infused via a second electronic device, where its administration set was connected to the y-port of the maintenance fluid administration set. Both fluids were administered simultaneously.

Blood samples were obtained and physiological variables recorded at fixed time-points during the haemorrhage and resuscitation phase. After the 120-minute resuscitation phase blood was sampled; the monitoring equipment and catheters, except the jugular catheter, were removed, and the thorax was radiographed. The carotid artery catheter was removed and digital pressure was applied until a stable clot was formed. If a stable clot did not form within 15 minutes then a haemostatic absorbable mesh (BloodStop iX; Life Science Plus; USA, CA) was applied using digital pressure for 10 minutes, or until haemostasis was achieved. Then the cut-down incision site was sutured using a two-layer closure technique with absorbable suture material (MonoPlus 5/0; B Braun). The cat was then transferred to the intensive care unit for recovery and overnight observation without the need for any further interventions or treatments. The next day, blood was sampled and the thorax radiographed before the cat was transferred back to the cattery. The cats were monitored for two days after the procedures by daily clinical examinations and observation of their social, drinking and eating behaviours. All cats were rehomed though an adoption process one month after concluding the study; and none of the cats had any untoward effects or organ (specifically, lung, renal and liver) injury or damage resulting from the study procedures.

3.3.3 Data collection and analysis

The total blood loss at each time point was cumulative and included the controlled haemorrhage volume (not applicable in control) and all blood samples for profiling. The blood loss in control was defined as

mild haemorrhage (<10 ml kg^{-1}). A severe haemorrhage was defined when more than 22 mL kg^{-1} (> 40% assumed circulating volume) of blood was lost. The total volumes of fluids that were administered during resuscitation were used for reporting because all cats were administered maintenance fluids during all treatments and that volume (10 mL kg^{-1} over 120 minutes) was considered negligible. Data were collected in the form of thoracic radiographs, blood sampling (venous and arterial) and physiological measurements that were taken at various time points throughout the study procedures and various ratios and gradients were calculated, where applicable (**Table 3.1**).

Thoracic radiographs were taken using a mobile unit (Poskom diagnostic x-ray unit model PXP-40HF; Tube model D-124 Toshiba) which was set to 75 kV and 2.0 mAs for all exposures. Images were captured on electronic cassettes (24 x 30 cm; Fujifilm IP cassette type CC) and evaluated by an experienced veterinarian to identify lung oedema or pleural effusion using diagnostic imaging software (easyImage; IMV imaging).

Table 3.1 Data collection time points and ratios and equations used in this study where anaesthetised cats underwent a severe haemorrhage event and fluid resuscitation using either lactated Ringer's solution or 6% tetrastarch 130/0.4 to restore the intravascular volume or a sham control treatment.

		Data collection				
Time period	**Physiological**	**Haematology**	**Biochemistry**	**Electrolyte**	**Blood gas**	**Radiographs**
One week prior to treatment						
Heath check (HC)	TPR	X	X	X		
Day of treatment						
-15	Full	X	X	X	X	X
-10	Full					
-5	Full					
-1	Full					
0	Full*	X	X	X	X	
30	Full*	X	X	X	X	
60	Full*	X	X	X	X	
90	Full*	X	X	X	X	
120	Full*	X	X	X	X	X
Next day after treatment						
Next day (NDay)	TPR	X	X	X		X

TPR: temperature-pulse-respiration; Full: complete data set of heart rate, respiratory rate, direct arterial blood pressure, end-tidal carbon dioxide measurement, oesophageal temperature and pulse oximetry; -15: baseline measurements; -10, -5, -1: time intervals during the haemorrhage phase of data collection; 0: time point after haemorrhage and immediately before starting the resuscitation phase; 30, 60, 90, 120: time intervals during the resuscitation phase; Full*: physiological parameters were captured at 10 minute intervals from time 0 to 120; X: when data was collected.

Ratios and equations	
Ratio or equation	**Comments**
Vgain	Vgain = EBV((H0 – Hf)/H0); modified Gross equation (Gross 1983) Where Vgain is the volume gained; EBV is the estimated initial blood volume prior to the resuscitation phase (55 ml/kg minus the cumulative blood loss volume; Groom et al. 1965 and Mott 1968); H0 is the initial haematocrit; and Hf is the final haematocrit. The volume gained was divided by the total volume infused to estimate the percentage of administered fluid remaining within the intravascular compartment which was then plotted on a graph over time.
Ht/Alb	Haematocrit (L L^{-1}) to serum albumin concentration (g L^{-1}) ratio
Na:K	Serum sodium (mmol L^{-1}) to serum potassium (mmol L^{-1}) ratio

Na-Cl	Serum sodium (mmol L^{-1}) to serum chloride (mmol L^{-1}) difference
Cl:Na	Serum chloride (mmol L^{-1}) to serum sodium (mmol L^{-1}) ratio
PCO_2(a-E′)	Arterial to end-tidal carbon dioxide gradient. Calculated by subtracting the end-tidal carbon dioxide value from the arterial partial pressure of carbon dioxide value, both taken at the same time point.
PO_2(A-a)	Aa gradient, or alveolar to arterial partial pressure of oxygen gradient. The partial pressure of alveolar oxygen was calculated using the following formula: $PAO_2 = FiO_2(Pbar-P_{H2O}) – (PaCO_2/RQ)$ Where PAO_2 is the partial pressure of alveolar oxygen; FiO_2 is the fraction of inspired oxygen (% value as a decimal; set at 0.96); Pbar is the barometric air pressure [mean barometric pressure 661 mmHg; altitude 1250 meters]; P_{H2O} is the saturated vapour pressure of water at 37°C which was set to 47 mmHg; $PaCO_2$ is the arterial partial pressure of carbon dioxide (mmHg); and RQ is the respiratory quotient set at 0.8.
CaO_2	CaO_2 = (Hufner's constant x Hb x SaO_2) + (0.003 x PaO_2) Where CaO_2 is the arterial oxygen content (mL dL^{-1}); Hufner's constant set at 1.39 mL dL^{-1} for cats (Herrmann & Haskins 2005); Hb is the haemoglobin concentration (g dL^{-1}); SaO_2 is the arterial haemoglobin saturation of haemoglobin (% value as a decimal); PaO_2 is the arterial partial pressure of oxygen (mmHg).
CvO_2	CvO_2 = (Hufner's constant x Hb x SvO_2) + (0.003 x PvO_2) Where CvO_2 is the venous oxygen content (mL dL^{-1}); Hufner's constant set at 1.39 mL dL^{-1} for cats (Herrmann & Haskins 2005); Hb is the haemoglobin concentration (g dL^{-1}); SvO_2 is the venous haemoglobin saturation of haemoglobin (% value as a decimal); PvO_2 is the venous partial pressure of oxygen (mmHg).
OER	OER = ($CaO_2 – CvO_2$)/CaO_2 x 100% Where OER is the oxygen extraction ratio; CaO_2 is the arterial oxygen content (mL dL^{-1}); CvO_2 is the venous oxygen content (mL dL^{-1}).
PF ratio	Arterial partial pressure of oxygen (mmHg) is divided by the fraction of inspired oxygen (% value as a decimal) to calculate the PF ratio

Prior to collection of arterial or venous blood samples via the catheters for laboratory analyses, an initial 1.5 ml volume was drawn and discarded to ensure truly representative samples were collected. Venous blood samples were withdrawn from the jugular catheter into a syringe and immediately decanted into various vacuum storage tubes (BD Vacutainer tube, BD), in the following order: citrate (coagulation study not reported on here), serum then Ethylenediaminetetraacetic acid (EDTA). The stored blood was submitted immediately to the onsite laboratory for analyses as follows: (1) haematology (EDTA tube), (2) serum biochemistry (serum tube - serum albumin, blood urea nitrogen [BUN], creatinine, glucose, lactate) and (3) electrolytes (serum tube - sodium, chloride, potassium, phosphorus, total magnesium, ionised calcium) using daily-calibrated machines by experienced veterinary clinical pathologists. For blood gas analyses, arterial and venous blood was withdrawn into heparinised syringes (BD A-line; BD) and sent to the onsite laboratory for analysis within 10 minutes of collection, using a daily-calibrated machine (mean barometric pressure 661 mmHg; altitude 1250 meters; interpreted at a fixed temperature of 37°C).

The volume of resuscitation fluid gained within the intravascular compartment over time during the resuscitation phase was estimated using a modified version of the Gross equation (**Table 3.1**; Gross 1983).

Data were assessed for equal variance and distribution by examining histograms and evaluating descriptive statistics. The duration of the haemorrhage phase was compared using a one-way analysis of variance. Endpoints of the haemorrhage phase were described using count data. Physiological, haematological, serum biochemical, electrolyte and blood gas data were compared among treatments over time using a general linear mixed model (main effects of treatment and time, main outcome was the interaction treatment x time) and significant findings underwent post-hoc Tukey pairwise comparisons. Model fits were assessed by visually inspecting residual plots. Data of all variables were tabulated among treatments over time alongside the cumulative blood loss (control haemorrhage + blood sample volumes) and fluid volumes (reported in ml kg^{-1}). Significant variables (based on outcomes of described statistical tests) were further evaluated to determine potential volume-endpoints. The volume-endpoints were defined as a change of a variable value outside of an expected clinical range for a domestic cat. Cats were identified as either fluid overloaded (yes) by the presence of any of the following: 1) radiographic lung patterns and pleural effusions, 2) facial oedema (generalised or intermandibular region), 3) thoracic or pelvic limb oedema, and 4) serous or serosanguinous fluid discharge from the respiratory tract at any time point in the study (Thomovsky et al. 2016); or as not fluid overloaded (no) in the absence of these findings, determined at time point 120 minutes. Variables that fulfilled the volume-endpoint definition were plotted on receiver operating characteristic (ROC) curves, area under the curve (AUC) and the Youden index, using the dichotomous outcomes (yes: fluid overloaded; no: not fluid overloaded) to determine optimal cut-off points to guide volume resuscitation. Data were analysed using commercially available software (MiniTab 18.1; Minitab Inc; and MedCalc) and significance interpreted at $p < 0.05$. Data are reported as mean (standard deviation; SD) and only p values for the interaction treatment x time are reported.

3.4 Results

During the haemorrhage phase, the sham haemorrhage time was 15.2 (± 0.8) minutes and similar to the controlled haemorrhages of LRS (17.5 ± 2.3 minutes) and Vol (18.6 ± 4.4 minutes) ($p = 0.137$). The maximum blood withdrawal volume endpoint was applied 4 times (in 2 LRS and 2 Vol treatments), while the mean arterial blood pressure endpoint was applied for the remainder of times (8 times in 4 LRS and

4 Vol treatments). Fluid overload was not detected in control but were present in LRS (n = 5) and Vol (n = 3).

During LRS and Vol treatments, and only during the haemorrhage phase, heart rate increased (p = 0.014) while systolic arterial blood pressure decreased (p = 0.014). These variables returned to baseline values within 10 minutes of resuscitation (**Figure 3.1**). The other physiological variables (except the mean and diastolic arterial blood pressures) remained unchanged during all treatments (**Table 3.2**).

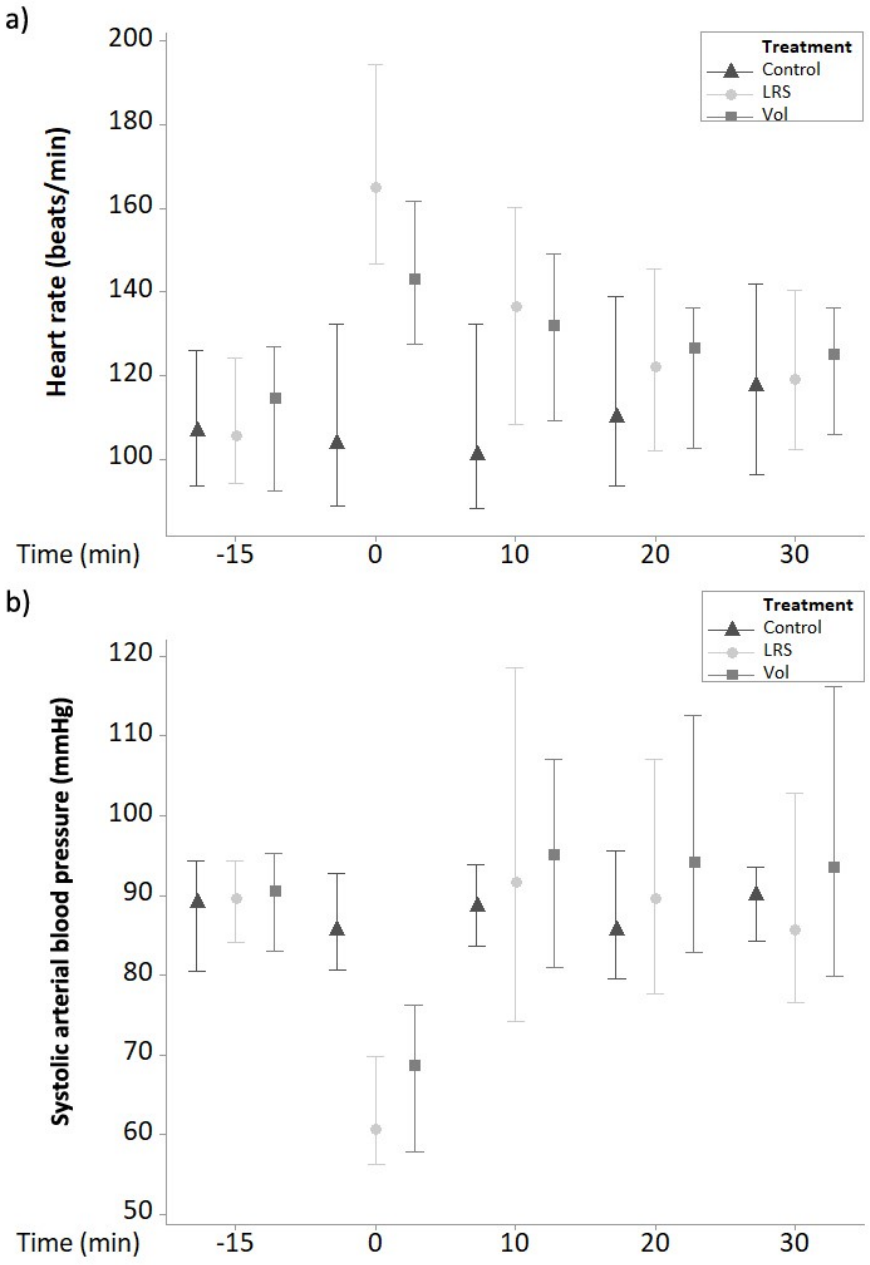


Figure 3.1 Confidence interval plots (95%) of the heart rate (a) and systolic arterial blood pressure (b) recorded over time in isoflurane in oxygen anaesthetised cats undergoing three treatments. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled, severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusions for resuscitation over 120 minutes. Measurements were taken before haemorrhage (-15), after haemorrhage (0) and then at 10-minute intervals (10, 20, 30) during fluid resuscitation.

Table 3.2 Physiological variable values of isoflurane in oxygen anaesthetised cats that underwent a severe haemorrhage (> 22 mL kg^{-1}) event and fluid resuscitation using either lactated Ringer's solution (LRS) or 6% tetrastarch 130/0.4 (Vol) to restore the intravascular volume or a control treatment of mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation. Physiological data was collected at baseline (Time -15), during haemorrhage (-10, -5, -1), after haemorrhage immediately before fluid resuscitation (Time 0) and during resuscitation at 30-minute intervals (Time 30, 60, 90, 120).

Time	**Treatment**	**Cumulative blood loss**		**Heart rate**		**SAP**		**MAP**		**DAP**		**Respiratory rate**		**End-tidal carbon dioxide**		**Temperature**		**SpO_2**	
(min)	(ml kg^{-1})	(ml kg^{-1})		(beats min^{-1})		(mmHg)		(mmHg)		(mmHg)		(breaths min^{-1})		(mmHg)		(°C)		(%)	
	Control	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
-15	(-)	1.3	0.3	110	16	87	7	69	8	55	9	14	4	41	6	36.4	0.6	99	1
-10	(-)	-	-	112	16	89	4	69	7	56	9	14	4	40	6	36.4	0.6	99	1
-5	(-)	-	-	112	17	90	4	69	7	56	9	14	4	41	6	36.4	0.6	100	0
-1	(-)	-	-	111	18	88	5	68	6	55	7	14	4	41	7	36.4	0.6	100	0
0	(0)	4.5	1.1	111	21	87	6	64	4	52	5	14	5	41	7	36.3	0.6	99	1
20	(0)	-	-	113	21	88	6	67	5	55	7	16	7	41	7	36.2	0.6	99	1
40	(0)	4.7†	1.1	123	21	91	6	73	8	59	7	21	10	40	8	36.3	0.7	100	1
60	(0)	6.8	1.6	132	23	102	14	83	14	67	12	26	9	39	8	36.6	0.7	99	1
80	(0)	-	-	142	24	98	16	79	18	64	15	27	9	39	8	36.8	0.7	100	1
100	(0)	8.1‡	1.9	140	27	94	12	74	15	59	14	29	9	38	7	37.0	0.7	100	0
120	(0)	10.2	2.3	144	34	95	14	74	16	59	15	30	11	37	7	37.1	0.8	100	1
	LRS	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
-15	(-)	1.3	0.3	110	14	89	5	71	7	56	7	14	5	44	4	36.6	0.6	100	0
-10	(-)	7.8	2.0	121	18	74	7	53	9	43	8	16	6	41	7	36.6	0.5	99	1
-5	(-)	14.4	3.7	152*	26	76	13	58	17	49	14	17	7	39	6	36.7	0.6	100	0
-1	(-)	22.1	5.3	158*	26	70*	15	48	9	40	9	18	6	37	5	36.7	0.5	99	1
0	(0)	22.3	7.3	171*	23	63*	6	45	5	38	5	20	8	37	4	36.6	0.6	100	0
20	(20)	-	-	129	22	94	16	72	17	53	13	16	6	40	5	36.1	0.6	100	0
40	(40)	23.6†	7.6	124	21	91	12	68	14	51	12	15	4	41	3	36.0	0.6	99	1
60	(60)	25.8	8.1	129	24	96	15	74	18	57	16	18	9	40	3	36.0	0.6	100	0
80	(80)	-	-	154	32	103	21	85	23	70	23	22	12	37	3	36.0	0.6	100	1
100	(100)	27.1‡	8.5	162	33	110	30	90	28	74	25	25	11	35	4	36.2	0.6	100	0
120	(120)	29.3	9.0	174	29	110	28	90	25	72	22	26	11	35	5	36.3	0.5	100	0

Table 3.2 continues on next page

Table 3.2 continued

Time	Treatment	Cumulative blood loss		Heart rate		SAP		MAP		DAP		Respiratory rate		End-tidal carbon dioxide		Temperature		SpO_2	
(min)	(ml kg^{-1})	(ml kg^{-1})		(beats min^{-1})		(mmHg)		(mmHg)		(mmHg)		(breaths min^{-1})		(mmHg)		(°C)		(%)	
	Vol	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
-15	(-)	1.3	0.3	110	17	89	6	68	10	53	9	16	9	40	7	36.4	0.5	99	1
-10	(-)	7.9	2.1	122	28	75	10	51	11	41	10	16	9	38	6	36.3	0.5	100	0
-5	(-)	14.5	3.8	132	18	70*****	15	50	16	40	13	15	6	37	7	36.3	0.4	99	1
-1	(-)	19.9	5.5	139	15	66*****	8	45	8	36	7	13	5	37	8	36.2	0.5	99	1
0	(0)	21.6	4.7	145*****	16	67*****	9	45	5	36	4	14	6	37	7	36.2	0.6	100	1
20	(7)	-	-	124	17	96	13	70	15	54	14	16	7	39	7	36.1	0.5	100	1
40	(13)	23.4†	5.1	122	15	99	17	73	19	55	14	18	9	39	7	36.1	0.5	100	0
60	(20)	25.6	5.6	128	15	100	11	77	13	60	13	22	11	38	8	36.3	0.4	99	1
80	(27)	-	-	144	24	102	16	81	18	63	17	28	18	35	10	36.6	0.4	99	1
100	(33)	26.9‡	5.6	164	32	105	16	84	16	67	15	30	18	34	11	36.9	0.6	100	1
120	(40)	29.1	6.3	162	34	102	17	81	17	64	15	27	18	35	10	37.1	0.5	99	2

Footnote:

*****statistically significant ($p < 0.05$) result using a general linear mixed model and post-hoc Tukey pairwise comparisons (interaction: time x treatment).

†actual measurement taken at 30 minutes

‡actual measurement taken at 90 minutes

min: minute; SAP: systolic arterial blood pressure; MAP: mean arterial blood pressure; DAP: diastolic arterial blood pressure; SpO_2: peripheral oxygen-haemoglobin saturation.

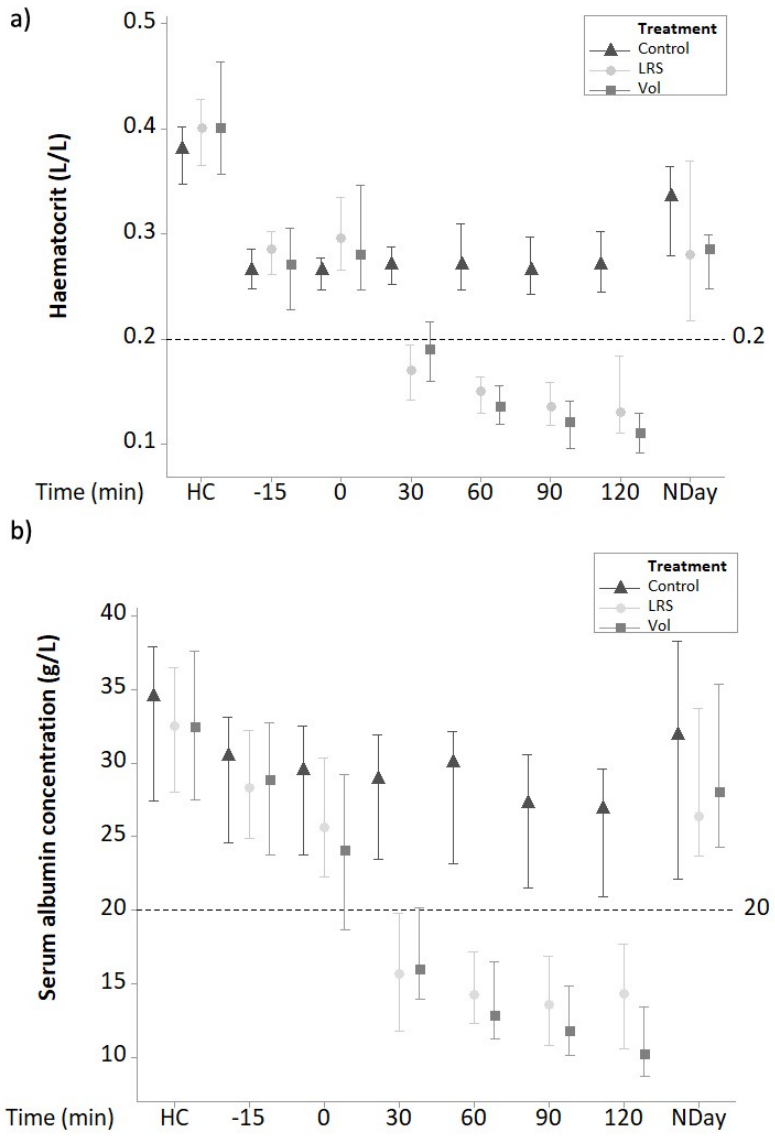


Figure 3.2 Confidence interval plots (95%) of the haematocrit (a) and serum albumin concentration (b) measured over time in isoflurane in oxygen anaesthetised cats undergoing three treatments. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. Measurements were taken during a health check (HC), before haemorrhage (-15), after haemorrhage (0) and then at 30-minute intervals (30, 60, 90, 120) during fluid resuscitation and the next day (NDay).

During the resuscitation phase, the haematocrit decreased (p < 0.001) from 0.17 to 0.15 L L^{-1} at 30 minutes to 120 minutes during LRS, and from 0.19 to 0.11 L L^{-1} during Vol; but did not change during the control sham resuscitation (**Figure 3.2**). Red cell count decreased continuously (p < 0.001) from 30 minutes to 120 minutes during both treatments. The mean corpuscular volume, mean corpuscular haemoglobin concentration, leukogram and thrombogram remained unchanged during all treatments (**Table 3.3**). The serum albumin decreased during LRS and Vol in a similar manner from 30 to 120 minutes, but did not change in the control (p < 0.001). Serum albumin shifted from 16 to 14 g L^{-1} for LRS and 17 to 11 g L^{-1} for Vol from 30 to 120 minutes (**Table 3.4** and **Figure 3.2**). Lactate rose from 30 to 120 minutes during LRS treatment only (p = 0.004). The other biochemical variables and the haematocrit to serum albumin ratio values remained unchanged during all treatments.

Table 3.3 Haematological values of isoflurane in oxygen anaesthetised cats that underwent a severe haemorrhage (> 22 mL kg^{-1}) event and fluid resuscitation using either lactated Ringer's solution (LRS) or 6% tetrastarch 130/0.4 (Vol) to restore the intravascular volume or a control treatment of mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation. Blood was collected one week before treatments during health checks (HC), at baseline (Time -15), after haemorrhage immediately before fluid resuscitation (Time 0), during resuscitation at 30-minute intervals (Time 30, 60, 90, 120) and the next day after treatment (NDay).

Time	**Treatment**	**Cumulative blood loss**		**Haematocrit**		**Red cell count**		**MCV**		**MCHC**		**White cell count**		**Neutrophils**		**Lymphocytes**		**Thrombocytes**	
(min)	(ml kg^{-1})	(ml kg^{-1})		(L L^{-1})		(x10^{12} L^{-1})		(fL)		(g dL^{-1})		(x10^{9} L^{-1})		(x10^{9} L^{-1})		(x10^{9} L^{-1})		(x10^{9} L^{-1})	
	Control	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
HC	(-)	-	-	0.38*	0.03	8.4*	0.7	44.9	2.5	33.5	1.0	14.3	12.9	9.9	10.6	3.6	2.0	346	111
-15	(-)	1.3	0.3	0.27	0.02	6.0	0.7	45.1	2.8	32.3	1.2	8.9	6.0	5.3	3.8	3.0	2.1	331	61
0	(0)	4.5	1.1	0.26	0.01	5.9	0.7	44.8	2,8	32.1	1.1	8.5	5.6	5.3	3.6	2.6	1.6	336	70
30	(0)	4.7	1.1	0.27	0.02	6.0	0.5	45.0	3.0	31.9	0.5	9.2	7.5	6.1	5.1	2.3	1.4	306	90
60	(0)	6.8	1.6	0.28	0.03	6.3	1.0	44.6	2.9	33.3	1.6	9.9	6.4	7.4	5.5	1.7	1.1	300	46
90	(0)	8.1	1.9	0.27	0.03	6.1	1.0	44.8	2.6	31.9	0.7	10.6	6.4	8.4	5.4	1.6	0.7	175	81
120	(0)	10.2	2.3	0.27	0.03	6.1	0.8	44.7	2.4	32.4	1.4	10.7	5.6	8.5	5.0	3.8	6.2	264	134
NDay	(0)	-	-	0.32*	0.04	7.1	1.0	45.8	2.8	32.6	1.3	15.2	11.4	10.5	11.6	4.0	1.5	281	63
	LRS	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
HC	(-)	-	-	0.40*	0.03	8.9*	0.9	44.8	1.8	33.2	1.5	12.9	5.7	8.6	4.9	3.5	0.8	367	71
-15	(-)	1.3	0.3	0.28	0.02	6.2	0.7	45.0	3.6	31.7	1.1	7.6	2.4	4.2	2.0	3.0	1.7	336	49
0	(0)	22.3	7.3	0.30	0.03	6.8	1.0	44.7	2.3	32.4	1.0	6.1	1.9	3.7	2.3	2.4	0.7	255	90
30	(30)	23.6	7.6	0.17*	0.02	3.8*	0.7	45.1	2.5	31.3	1.0	5.1	1.6	2.9	1.6	1.8	0.5	228	57
60	(60)	25.8	8.1	0.15*	0.02	3.3*	0.4	44.4	2.6	31.5	0.9	6.9	2.2	3.9	1.8	2.5	1.4	174	85
90	(90)	27.1	8.5	0.14*	0.02	3.1*	0.4	44.2	2.5	31.4	0.9	8.9	3.3	6.3	2.7	2.1	1.0	192	42
120	(120)	29.3	9.0	0.15*	0.04	3.3*	0.7	44.1	2.0	31.7	1.5	8.9	3.0	6.1	3.0	2.4	2.3	189	40
NDay	(-)	-	-	0.29	0.07	6.6	1.7	44.4	2.2	32.2	1.2	10.0	2.8	5.9	2.7	3.6	2.0	245	62
	Vol	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
HC	(-)	-	-	0.41*	0.05	9.1*	1.6	45.1	3.5	32.9	1.3	11.6	7.6	7.8	6.3	2.8	1.0	320	88
-15	(-)	1.3	0.3	0.27	0.04	5.9	1.0	44.9	3.8	32.0	1.2	7.7	3.3	4.6	2.6	2.5	1.3	343	45
0	(0)	21.6	4.7	0.30	0.05	6.6	1.3	45.1	4.3	32.0	1.3	7.1	5.1	4.2	3.2	2.5	1.9	328	58
30	(10)	23.4	5.1	0.19*	0.03	4.2*	0.7	45.0	4.1	30.6	3.4	6.9	5.3	4.3	4.0	2.3	2.0	268	57
60	(20)	25.6	5.6	0.14*	0.02	3.1*	0.5	44.6	3.9	31.6	1.4	7.9	6.6	5.2	4.5	2.1	2.0	191	63
90	(30)	26.9	5.6	0.12*	0.02	2.7*	0.5	44.8	4.1	31.6	1.4	8.5	5.5	6.6	4.5	1.4	1.1	154	61
120	(40)	29.1	6.3	0.11*	0.02	2.5*	0.5	45.0	4.1	31.4	1.5	7.7	4.7	6.1	4.2	1.1	0.6	132	60
NDay	(-)	-	-	0.27	0.02	5.9	0.6	46.0	3.6	32.4	1.0	13.8	11.0	8.2	7.4	5.2	3.6	246	49

Footnote:

*statistically significant ($p < 0.05$) result using a general linear mixed model and post-hoc Tukey pairwise comparisons (interaction: time x treatment).

min: minute; MCV: mean corpuscular volume; MCHC: mean corpuscular haemoglobin concentration; HC: health check done one week before treatments; NDay: next day after treatments.

Table 3.4 Biochemistry values of isoflurane in oxygen anaesthetised cats that underwent a severe haemorrhage (> 22 mL kg^{-1}) event and fluid resuscitation using either lactated Ringer's solution (LRS) or 6% tetrastarch 130/0.4 (Vol) to restore the intravascular volume or a control treatment of mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation. Blood was collected one week before treatments during health checks (HC), at baseline (Time -15), after haemorrhage immediately before fluid resuscitation (Time 0), during resuscitation at 30-minute intervals (Time 30, 60, 90, 120) and the next day after treatment (NDay).

Time (min)	**Treatment** (ml kg^{-1})	**Cumulative blood loss** (ml kg^{-1})		**Serum albumin** (g L^{-1})		**BUN** (mmol L^{-1})		**Creatinine** (µmol L^{-1})		**Glucose** (mmol L^{-1})		**Lactate** (mmol L^{-1})		**Ht/Alb**	
	Control	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
HC	(-)	-	-	33	5	8.4	1.6	126	31	5.6	1.0	2.1	0.2	1.2	0.1
-15	(-)	1.3	0.3	29	4	8.4	1.0	124	20	6.4	1.3	1.4	0.3	0.9	0.1
0	(0)	4.5	1.1	28	4	8.5	0.9	123	21	6.3	1.5	1.5	0.3	0.9	0.1
30	(0)	4.7	1.1	28	4	8.4	1.0	121	20	6.3	1.4	1.4	0.4	1.0	0.2
60	(0)	6.8	1.6	28	4	8.2	0.8	118	21	6.6	1.5	1.7	0.5	1.0	0.1
90	(0)	8.1	1.9	26	4	8.1	1.0	119	20	7.0	2.3	1.8	0.3	1.1	0.2
120	(0)	10.2	2.3	25	4	8.1	1.1	118	20	7.1	2.0	1.9	0.3	1.1	0.1
NDay	(0)	-	-	30	8	7.2	1.5	111	17	6.5	1.2	3.3	1.8	1.1	0.2
	LRS	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
HC	(-)	-	-	32	4	8.5	1.5	123	21	6.3	0.5	3.3	0.8	1.2	0.2
-15	(-)	1.3	0.3	29	4	8.1	1.3	111	14	6.5	2.2	2.0	0.7	1.0	0.2
0	(0)	22.3	7.3	26	4	8.5	1.0	125	15	7.2	2.1	1.9	0.7	1.2	0.3
30	(30)	23.6	7.6	16*	4	7.1	1.6	92	21	6.2	2.6	4.7*	1.6	1.1	0.2
60	(60)	25.8	8.1	15*	2	7.0	1.2	91	9	5.9	2.4	3.4	1.8	1.0	0.1
90	(90)	27.1	8.5	14*	3	6.6	1.2	87	11	5.3	2.2	3.9*	1.7	1.1	0.1
120	(120)	29.3	9.0	14*	3	6.4	1.0	88	13	5.3	1.6	3.4	0.5	1.1	0.2
NDay	(-)	-	-	29	5	6.2	0.5	99	17	5.9	0.7	2.0	0.5	1.0	0.3
	Vol	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
HC	(-)	-	-	33	5	8.1	2.7	121	18	6.5	1.8	2.1	0.5	1.3	0.2
-15	(-)	1.3	0.3	28	4	8.2	1.7	124	21	6.5	0.8	1.6	0.6	1.0	0.2
0	(0)	21.6	4.7	24	5	8.1	1.3	125	16	6.6	1.2	2.2	1.3	1.3	0.3
30	(10)	23.4	5.1	17*	3	7.9	1.7	118	17	6.8	1.1	1.4	0.3	1.1	0.2
60	(20)	25.6	5.6	14*	3	7.8	1.7	113	16	6.7	1.0	1.1	0.3	1.0	0.2
90	(30)	26.9	5.6	13*	2	7.4	1.6	112	15	6.5	1.2	1.4	0.8	1.0	0.2
120	(40)	29.1	6.3	11*	2	7.4	1.5	110	13	7.0	1.0	1.3	0.3	1.0	0.3
NDay	(-)	-	-	30	5	6.8	1.8	102	16	7.2	1.7	2.8	0.8	0.9	0.2

Footnote:

*****statistically significant ($p < 0.05$) result using a general linear mixed model with post-hoc Tukey pairwise comparisons (interaction: time x treatment).

min: minute; BUN: blood urea nitrogen concentration; Ht/Alb: haematocrit to serum albumin ratio; HC: health check done one week before treatments; NDay: next day after treatments.

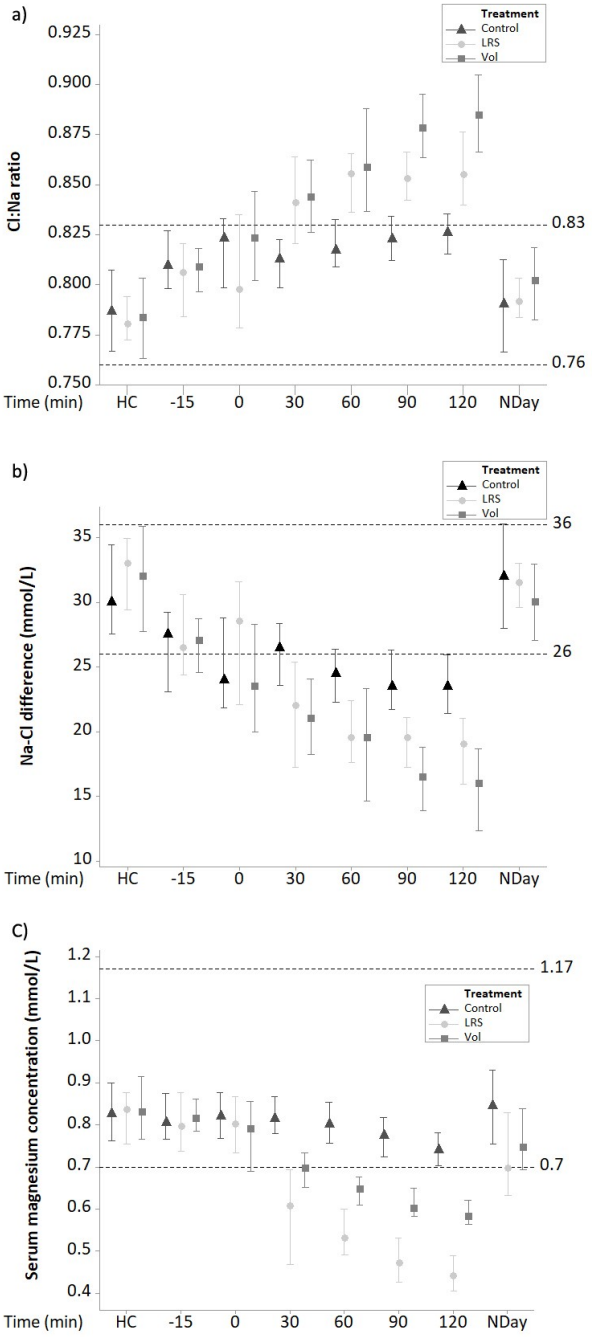


Figure 3.3 Confidence interval plots (95%) of the Cl:Na ratio (a), Na-Cl difference (b) and serum magnesium concentration (c) recorded over time in isoflurane in oxygen anaesthetised cats undergoing three treatments. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. Measurements were taken during a health check (HC), before haemorrhage (-15), after haemorrhage (0) and then at 30-minute intervals (30, 60, 90, 120) during fluid resuscitation and the next day (NDay). Where: Na-Cl: serum sodium to chloride concentration difference; Cl:Na: serum chloride to sodium ratio

The total magnesium decreased significantly from 30 to 120 minutes for LRS and Vol but not for the control (p < 0.001). For LRS, magnesium dropped from 0.58 to 0.45 mmol L^{-1} and for Vol 0.69 to 0.59 mmol L^{-1} (**Table 3.5**). The other electrolytes remained unchanged in all treatments. However, the chloride to sodium ratio (p < 0.001) increased and sodium-chloride difference (p = 0.003) decreased significantly in LRS and Vol. The sodium to potassium ratio remained unchanged in all treatments. The significant electrolyte shifts are depicted in **Figure 3.3**. The electrolytes were the only variables that demonstrated the potential for one to determine volume load, and cut-off values could be determined (**Table 3.6**).

Table 3.5 Electrolyte values of isoflurane in oxygen anaesthetised cats that underwent a severe haemorrhage (> 22 mL kg^{-1}) event and fluid resuscitation using either lactated Ringer's solution (LRS) or 6% tetrastarch 130/0.4 (Vol) to restore the intravascular volume or a control treatment of mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation. Blood was collected one week before treatments during health checks (HC), at baseline (Time -15), after haemorrhage immediately before fluid resuscitation (Time 0), during resuscitation at 30-minute intervals (Time 30, 60, 90, 120) and the next day after treatment (NDay).

Time (min)	**Treat** (ml kg^{-1})	**Cumulative BL** (ml kg^{-1})		**Na** (mmol L^{-1})		**Cl** (mmol L^{-1})		**K** (mmol L^{-1})		**P** (mmol L^{-1})		**Ca^{2+}** (mmol L^{-1})		**Mg** (mmol L^{-1})		**Na:K**		**Na-Cl** (mmol L^{-1})		**Cl:Na**	
	Control	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
HC	(-)	-	-	146	9	115	8	3.4	0.4	1.6	0.2	1.1	0.1	0.83	0.07	43	3	31	3	0.79	0.02
-15	(-)	1.3	0.3	139	8	113	5	3.5	0.4	2.0	0.2	1.1	0.1	0.82	0.05	40	4	26	3	0.81	0.01
0	(0)	4.5	1.1	137	6	112	3	3.6	0.4	2.0	0.2	1.1	0.1	0.82	0.05	38	4	25	3	0.82	0.02
30	(0)	4.7	1.1	137	5	111	4	3.8	0.4	2.0	0.2	1.1	0.1	0.82	0.04	36	3	26	2	0.81	0.01
60	(0)	6.8	1.6	136	4	111	3	3.9	0.6	1.9	0.2	1.0	0.1	0.81	0.05	36	5	24	2	0.82	0.01
90	(0)	8.1	1.9	136	5	112	3	3.9	0.5	1.8	0.2	1.0	0.1	0.77	0.04	35	4	24	2	0.82	0.01
120	(0)	10.2	2.3	135	5	112	3	3.8	0.4	1.8	0.2	1.0	0.1	0.74	0.04	36	4	24	2	0.83	0.01
NDay	(0)	-	-	152	3	120	2	4.1	0.6	1.6	0.2	1.2	0.0	0.84	0.08	37	4	32*	4	0.79	0.02
	LRS	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
HC	(-)	-	-	148	6	116	3	3.3	0.3	1.5	0.1	1.2	0.1	0.82	0.06	45	3	32*	3	0.78	0.01
-15	(-)	1.3	0.3	139	4	111	2	3.6	0.2	2.1	0.2	1.1	0.1	0.81	0.07	39	3	28	3	0.80	0.02
0	(0)	22.3	7.3	138	6	111	3	3.9	0.3	2.3	0.2	1.1	0.1	0.80	0.06	36	3	27	5	0.81	0.03
30	(30)	23.6	7.6	135	8	113	5	3.8	0.3	1.6	0.3	1.1	0.1	0.58*	0.11	36	4	21	4	0.84*	0.02
60	(60)	25.8	8.1	134	5	114	4	3.9	0.3	1.6	0.1	1.1	0.1	0.55*	0.05	35	3	20*	2	0.85*	0.01
90	(90)	27.1	8.5	132	4	112	3	3.8	0.3	1.5	0.1	1.0	0.1	0.48*	0.05	34	2	19*	2	0.85*	0.01
120	(120)	29.3	9.0	130	3	112	3	3.8	0.3	1.4	0.1	1.0	0.1	0.45*	0.04	35	2	19*	2	0.86*	0.02
NDay	(-)	-	-	152	2	120	1	3.6	0.1	1.4	0.2	1.2	0.0	0.73	0.09	42	1	31	2	0.79	0.01
	Vol	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
HC	(-)	-	-	147	9	115	7	3.5	0.4	1.5	0.1	1.1	0.1	0.84	0.07	43	4	32*	4	0.78	0.02
-15	(-)	1.3	0.3	138	4	112	3	3.5	0.2	2.2	0.1	1.1	0.1	0.82	0.04	39	3	27	2	0.81	0.01
0	(0)	21.6	4.7	137	9	113	6	3.9	0.3	2.1	0.2	1.1	0.1	0.77	0.08	35	4	24	4	0.82	0.02
30	(10)	23.4	5.1	136	6	115	5	3.6	0.2	1.9	0.1	1.0	0.1	0.69*	0.04	38	4	21	3	0.84*	0.02
60	(20)	25.6	5.6	137	6	118	2	3.7	0.3	1.8	0.1	1.1	0.1	0.64*	0.03	37	4	19*	4	0.86*	0.02
90	(30)	26.9	5.6	135	3	119	2	3.8	0.4	1.6	0.2	1.0	0.1	0.62*	0.03	36	4	16*	2	0.88*	0.02
120	(40)	29.1	6.3	135	7	120	5	3.8	0.6	1.6	0.2	1.0	0.1	0.59*	0.03	36	6	16*	3	0.89*	0.02
NDay	(-)	-	-	150	5	120	5	3.7	0.3	1.4	0.3	1.2	0.1	0.77	0.07	41	3	30	3	0.80	0.02

Footnote:

*statistically significant ($p < 0.05$) result using a general linear mixed model with post-hoc Tukey pairwise comparisons (interaction: time x treatment).

min: minute; Treat: treatment; BL: blood loss; Na: serum sodium concentration; Cl: serum chloride concentration; K: serum potassium concentration; P: inorganic phosphate concentration; Ca2+: ionised calcium concentration; Mg: magnesium concentration; Na:K: serum sodium to potassium ratio; Na-Cl: serum sodium to chloride concentration difference; Cl:Na: serum chloride to sodium ratio; HC: health check done one week before treatments; NDay: next day after treatments.

Table 3.6 Receiver operating characteristic (ROC) curves and the Youden index was used to determine optimal cut-off points to guide volume resuscitation of isoflurane in oxygen anaesthetised cats undergoing severe haemorrhage (> 22 mL kg^{-1}) and fluid resuscitation using a crystalloid (lactated Ringer's solution) or a colloid (6% tetrastarch 130/0.4; voluven) fluid administered at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively for 120 minutes.

Variable	ROC AUC	Youden index J	Associated criterion	Sensitivity	Specificity	+LR	-LR	PPV	NPP
Cl:Na	0.831	0.650	> 0.84	90	75	3.6	0.13	82	86
Na-Cl	0.778	0.556	< 21	100	56	2.3	0.0	69	100
Mg	0.975	0.889	< 0.57	89	100	4.0	0.11	100	90

Cl:Na: Chlorine to sodium ratio; Na-Cl: Sodium to chlorine difference; Mg: magnesium concentration; ROC AUC: Area under the ROC curve; +LR: positive likelihood ratio value; -LR: negative likelihood ratio value; PPV: positive predictive value; NPP: Negative predictive value

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Blood gas values, gradients (end-tidal carbon dioxide to arterial carbon dioxide; alveolar to arterial oxygen) and ratios (oxygen extraction ratio, arterial partial pressure to fraction inspired ratio) remained unchanged in all treatments (**Table 3.7**). Arterial ($p < 0.001$) and venous ($p < 0.001$) oxygen content decreased from 30 to 120 minutes in LRS and Vol but not control. Arterial oxygen content decreased from 9 to 8 ml dL^{-1} for LRS and from 9 to 5 ml dL^{-1} for Vol over the period from 30 to 120 minutes.

The resuscitation fluid volume gained at 120 minutes was approximately 13 ml kg^{-1} or 11% of the total volume infused for LRS and 16 ml kg^{-1} or 40% for Vol, respectively (**Figure 3.4**). The total LRS administered was at a 4.1:1 ratio to the total blood loss, while Vol was administered at a ratio of 1.4:1.

Overall, haematocrit, serum albumin, total magnesium, chloride to sodium ratio, sodium chloride difference demonstrated potential variables that could determine a volume resuscitation endpoint. These variables shifted in value out of normal clinical ranges for cats from 30 minutes of resuscitation onwards.

Table 3.7 Arterial blood gas values and oxygenation and ventilation indices of isoflurane in oxygen anaesthetised cats that underwent a severe haemorrhage (> 22 mL kg^{-1}) event and fluid resuscitation using either lactated Ringer's solution (LRS) or 6% tetrastarch 130/0.4 (Vol) to restore the intravascular volume or a control treatment of mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation. Blood was collected at baseline (Time -15), after haemorrhage immediately before fluid resuscitation (Time 0) and during resuscitation at 30-minute intervals (Time 30, 60, 90, 120).

Time	**Treatment**	**Cumulative blood loss**		**pH**		**$PaCO_2$**		**PaO_2** 100% O_2		**HCO_3(act)**		**HCO_3(std)**		**BE**		**TCO_2**	
(min)	(ml kg^{-1})	(ml kg^{-1})				(mmHg)		(mmHg)		(mEq L^{-1})		(mEq L^{-1})		(mEq L^{-1})		(mmol L^{-1})	
								Arterial blood gas values									
	Control	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
-15	(-)	1.3	0.3	7.32	0.09	38	11	367	41	18.4	2.0	18.5	0.9	-7.8	1.3	19.6	2.3
0	(0)	4.5	1.1	7.32	0.09	38	11	353	47	18.5	2.3	16.8	3.4	-7.7	1.5	19.7	2.6
30	(0)	4.7	1.1	7.33	0.09	36	9	373	25	18.3	2.3	18.8	2.0	-7.7	2.5	19.4	2.5
60	(0)	6.8	1.6	7.37	0.07	32	9	399	45	17.4	2.6	18.8	1.9	-8.0	2.5	18.4	2.8
90	(0)	8.1	1.9	7.39	0.08	32	8	393	39	18.5	2.0	19.9	1.8	-6.5	2.1	19.3	2.0
120	(0)	10.2	2.3	7.39	0.09	32	10	395	30	18.1	2.4	18.1	2.0	-7.0	2.6	19.1	2.6
	LRS	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
-15	(-)	1.3	0.3	7.29	0.06	41	6	363	29	18.9	1.8	18.5	1.7	-7.8	2.2	20.1	1.8
0	(0)	22.3	7.3	7.32	0.05	35	5	396	34	17.7	2.4	18.3	2.1	-8.5	2.8	18.7	2.5
30	(30)	23.6	7.6	7.32	0.03	38	3	396	35	19.2	1.1	19.1	1.1	-7.0	1.3	20.4	1.2
60	(60)	25.8	8.1	7.37	0.04	37	5	392	23	20.4	1.4	20.5	1.0	-5.2	1.4	21.4	1.6
90	(90)	27.1	8.5	7.40	0.04	34	6	359	38	20.5	1.5	21.1	0.8	-4.4	0.9	21.4	1.7
120	(120)	29.3	9.0	7.41	0.05	34	5	376	65	20.9	1.0	21.5	0.8	-3.8	1.0	21.9	1.2
	Vol	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
-15	(-)	1.3	0.3	7.28	0.08	43	5	390	46	19.3	1.8	18.7	2.4	-7.6	3.1	20.6	1.7
0	(0)	21.6	4.7	7.30	0.07	37	6	422	48	17.9	1.3	18.2	1.6	-8.7	1.8	19.0	1.4
30	(10)	23.4	5.1	7.31	0.07	38	7	413	35	18.5	1.9	18.6	2.0	-7.9	2.4	19.7	2.0
60	(20)	25.6	5.6	7.36	0.08	35	9	403	48	18.6	2.4	19.3	1.8	-7.0	2.1	19.7	2.6
90	(30)	26.9	5.6	7.38	0.11	34	10	392	22	18.6	2.0	19.6	2.3	-6.6	2.4	19.6	2.1
120	(40)	29.1	6.3	7.35	0.08	35	8	396	58	18.7	2.3	19.4	2.3	-6.8	2.5	19.8	2.4

Table 3.7 continues on next page

Table 3.7 continued

Time	Treatment	Cumulative blood loss		PCO_2(a- E′)		PO_2(A-a)		CaO_2		CvO_2		OER		PF ratio	
(min)	(ml kg^{-1})	(ml kg^{-1})		(mmHg)		(mmHg)		(ml dL^{-1})		(ml dL^{-1})		(%)			
							Oxygenation and ventilation indices								
	Control	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
-15	(-)	1.3	0.3	0	2	117	40	13	1	12	1	11	3	380	42
0	(0)	4.5	1.1	0	3	191	48	13	1	11	1	10	2	365	49
30	(0)	4.7	1.1	-1	3	171	25	13	1	11	1	12	3	388	27
60	(0)	6.8	1.6	-4	3	148	44	14	2	13	2	11	4	417	46
90	(0)	8.1	1.9	-3	2	156	35	13	1	11	1	14	6	410	40
120	(0)	10.2	2.3	-2	3	154	38	13	1	11	1	15	9	411	31
	LRS	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
-15	(-)	1.3	0.3	-3	2	178	29	13	1	11	2	16	8	376	31
0	(0)	22.3	7.3	-2	2	151	38	15	1	11	1	29*	9	410	34
30	(30)	23.6	7.6	-2	3	146	34	9*	1	7*	1	20	7	411	36
60	(60)	25.8	8.1	-3	3	153	20	8*	1	6*	1	17	8	406	23
90	(90)	27.1	8.5	-2	3	189	38	7*	1	6*	1	17	8	372	39
120	(120)	29.3	9.0	-1	2	172	64	8*	2	6*	2	19	10	391	68
	Vol	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***	***Mean***	***SD***
-15	(-)	1.3	0.3	2	4	149	46	13	2	11	2	14	8	404	50
0	(0)	21.6	4.7	0	5	124	50	14	2	10	2	33*	10	437	48
30	(10)	23.4	5.1	0	4	130	38	9*	2	8*	2	15	4	429	38
60	(20)	25.6	5.6	-2	2	144	50	7*	1	6*	1	17	5	419	51
90	(30)	26.9	5.6	0	5	156	24	6*	1	5*	1	16	7	407	24
120	(40)	29.1	6.3	0	6	148	56	6*	1	5*	1	19	7	413	62

Footnote:

*statistically significant ($p < 0.05$) result using a general linear mixed model with post-hoc Tukey pairwise comparisons (interaction: time x treatment).

min: minute; $PaCO_2$: arterial atrial pressure of carbon dioxide; PaO_2: arterial partial pressure of oxygen; HCO_3(act): actual bicarbonate ion concentration; HCO_3(std): standard bicarbonate ion concentration; BE: base excesses; TCO_2: total carbon dioxide concentration; PCO_2(a- E′): arterial to end-tidal gradient of carbon dioxide; PO_2(A-a): alveolar to arterial oxygen gradient; CaO_2: arterial content of oxygen; CvO_2: venous content of oxygen; OER: Oxygen extraction ratio; PF ratio: arterial partial pressure of oxygen to fraction of inspired oxygen ratio.

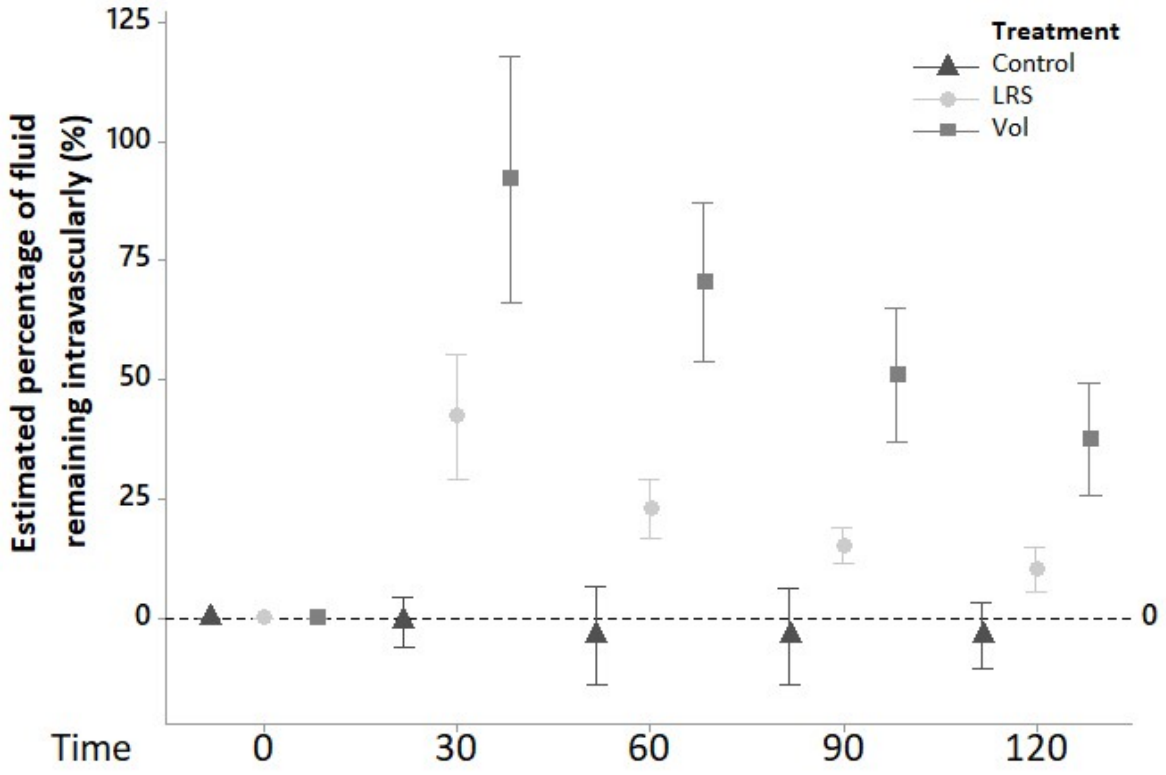


Figure 3.4 Confidence interval plots (95%) of the estimated percentage of resuscitation fluid remaining within the intravascular compartment over time in isoflurane in oxygen anaesthetised cats undergoing three treatments. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. Calculations were made at the time points after haemorrhage (0) and then at 30-minute intervals (30, 60, 90, 120) during fluid resuscitation.

3.5 Discussion

The present study demonstrates that LRS and Vol offered adequate volume replacement following haemorrhage-induced hypovolaemic hypotension. Fluid overload was detected in both fluids used and biomarkers proposed to be used as novel volume-endpoints could be identified. The volume-endpoints are a previously undescribed approach that can be used to guide fluid therapy and indicate when enough fluids have been administered to a patient during the resuscitation and optimisation phases of fluid therapy independent of their response to resuscitation. The volume-endpoints suggest that the administration of fluids at commonly recommended shock dose rates or ratio doses are excessive and not indicated in severely haemorrhaged anaesthetised cats. Chloride to sodium ratio and magnesium concentration appear to be good volume-endpoint biomarkers; but not heart rate, blood pressure, haematocrit or serum albumin concentration.

We recommend an initial resuscitation dose of 30 ml kg^{-1} or 10 ml kg^{-1} for LRS or Vol, respectively, over 15 to 20 minutes for cats that have sustained an acute controlled intraoperative haemorrhage of approximately 24 ml kg^{-1}, based on the electrolyte derived volume-endpoints. These fluid doses are similar to those administered to trauma cats, which receive an isotonic crystalloid (33.8 ± 19.3 ml kg^{-1}) or colloid (9.5 ± 5.3 ml kg^{-1}) during initial resuscitation effects (Wehausen et al. 2011). Furthermore, the ratio of administration was approximately 1.25:1 and 0.4:1 for LRS and Vol, respectively. These ratios are significantly lower than the 3:1 and 1:1 ratios for crystalloid and colloid replacement, respectively, that have been advocated for decades. Our interpretation of the volume-endpoints and the recommended initial doses of fluids are consistent with other investigators who have challenged these ratios (Hahn 2013; Fodor et al. 2019). Recent rat-based model research suggests that a 1:1 ratio should be used for isotonic crystalloid resuscitation; and they found that the rats had adequate compensatory capacity and no pulmonary oedema (Fodor et al. 2019). Also, if isotonic crystalloids are administered immediately after haemorrhage, then only a 1.6:1 ratio is required to restore the intravascular volume (Hahn 2013). These lower than expected dose ratios could be the origin of the sentiment that cats are not fluid tolerant and that caution is advised when resuscitating a cat. However, Hahn (2013) reviewed animal (rats and pigs) haemorrhage and resuscitation models and found that the ratios for crystalloids and colloids are indeed lower than recommended. Therefore, cats are perhaps similar in response to

fluid therapy as other animals and that unnecessary liberal fluid therapy being advocated in the past has led us to believe that they are not a fluid tolerant species.

To identify volume-endpoints, fluid overload was intentionally caused in healthy cats undergoing severe haemorrhage. Fluid overload is not an easy condition to treat and in healthy cats the treatment of stopping fluid administration and allowing time in a low stress environment resolved the overt fluid overload within 24 hours. Furthermore, the cats tolerated the severe haemorrhage and liberal fluid therapy without organ (lung, renal and liver) injury or damage, even a month after the conclusion of the study. Telephonic follow up with the adopter owners (at time of writing; one year after study treatments) confirmed that all of the cats are healthy without untoward effects of the study. This evidence suggests that healthy cats can tolerate severe haemorrhage and liberal fluid resuscitation without adverse long-term effects. Furthermore, we speculate that healthy cats can cope with liberal fluid therapy and that they are likely tolerant of fluids if administered in excess accidentally. Additionally, despite low haematocrit (lower than recommended transfusion triggers: Barfield & Adamantos 2011; Kisielewicz & Self 2014), decreased oxygen-carrying capacity and radiographic evidence of lung oedema there were no overt indications, oxygen debt, increased oxygen extraction and gas diffusion impairment [normal PO_2(A-a) gradient], contrary to what we expected to find (Thomovsky et al. 2016). It suggest that the oxygen demand did not exceed oxygen delivery and no oxygen debt accumulated, similar to what has been described in haemodynamically stable anaemic human patients (Antonelli et al. 2007; Napolitano et al. 2009). The haematocrit and serum albumin concentration in the treatments where controlled haemorrhage occurred was very low during the fluid resuscitation, but returned to approximately the same value by the next day as those values measured immediately after the controlled haemorrhage. This observation suggests that the decrease in the haematocrit and serum albumin concentrations was mostly attributed to the fluids that were being administered and that the cats could shed the excess fluids within 24 hours. Furthermore, the increase in haematocrit immediately post haemorrhage was likely because of the reserve blood volume entering circulation from the splenic organs (Greenway & Lister 1974) as no evidence of erythrocyte regeneration was evident (in the form of reticulocytosis). The decrease in serum albumin concentration immediately post haemorrhage was likely because of the loss of whole blood, and unlike reserve erythrocytes circulating in low flow organs (Greenway & Lister 1974), there was no movement of albumin from the interstitial space back into the intravascular compartment.

When fluids were administered after the haemorrhage, the Cl:Na ratio steadily increased as the volume of resuscitation fluids increased over time, and the ratio shifted past the reference interval (0.76 – 0.83) for cats (Goggs et al. 2017). The Cl:Na ratio for LRS was 0.85 (112 mmol L^{-1}: 131 mmol L^{-1}) and 1.0 (154 mmol L^{-1}: 154 mmol L^{-1}) for Vol. We theorise that the fluids were replacing the lost plasma volume and therefore, over the 120-minute resuscitation period, the electrolyte concentrations were predominantly influenced by the fluid rather than homeostatic functions of volume balance; therefore, suggesting that the Cl:Na ratio could be used as a resuscitation fluid volume endpoint. Most fluid used during resuscitation have a Cl:Na ratio > 0.85, however, few, like Normosol-R (Hospira; Lake Forest, IL, USA), have a Cl:Na ratio of 0.7 (98 mmol L^{-1}: 140 mmol L^{-1}) and contain magnesium (3 mmol L^{-1}). We speculate that if Normosol-R is used, then the Cl:Na ratio should decrease to < 0.76 (lower interval limit for cats). Therefore, we suspect that this volume-endpoint should also predict that enough Normosol-R has been administered to restore volume after severe haemorrhage, but further research is required to confirm our speculation. The Na-Cl difference also demonstrated a reliable trend, however, the control group also demonstrated differences < 26 mmol/L, and therefore we suspect that the electrolyte shifts during general anaesthesia may contribute to this shift and not only the resuscitation fluids. The magnesium concentration reliably decreased over time when the resuscitation fluids were administered and not in control; this effect was likely due to simple dilution because the resuscitation fluids used in this study do not contain magnesium (Whittaker et al. 2018). We speculate that this may be true for all resuscitation fluids that do not contain magnesium.

Haematocrit and serum albumin concentration decreased when the resuscitation phase commenced, which was expected, because of the fluid administration and compensatory fluid shifts (Napolitano et al. 2009). The predictable change in these variable values makes these variables less insightful endpoints for volume resuscitation in cats experiencing acute severe haemorrhage compared to the chloride to sodium ratio, for example. The haematocrit values were lower than frequently cited transfusion triggers (Barfield & Adamantos 2011; Kisielewicz & Self 2014). In the present study, cats survived the low haematocrit without the need for fresh whole blood transfusions, likely because they sustained uncomplex acute haemorrhage and no apparent increase in oxygen demand and augmented cardiac output because of the resuscitation fluids.

The study had notable limitations. The sample size is small and thus had the power to reliably detect only large effects. Furthermore, the observations herein will assist future researchers in estimating

adequate samples sizes to validate our findings under various clinical conditions. The study was conducted in anaesthetised cats under controlled conditions. Therefore, extrapolation of our findings to trauma or awake patients or those receiving different drugs during anaesthesia will require further investigation.

3.6 Conclusions

Biomarkers for volume-endpoints for haemorrhage-induced hypovolaemic hypotension in anaesthetised cats were identified. Overall, physiological variables, haematocrit or serum albumin concentration are not good indicators of circulating volume restoration compared to chloride to sodium ratio and magnesium concentration. The chloride to sodium ratio (> 0.84) might be a useful volume-endpoint compared to magnesium concentration (< 0.57 mmol/L) because they are more readily measured in clinical practice. Finding the ideal biomarker to identify that an adequate fluid dose has been administered during resuscitation of commonly used intravenous fluids will improve the overall safety of their administration and prevent fluid overload in cats.

CHAPTER 4

Blood acid-base analysis during crystalloid and colloid fluid resuscitation in haemorrhage-induced hypovolemic hypotensive anaesthetised cats

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4.1 Abstract

Objective To compare the variables used for Henderson-Hasselbalch, Stewart and semi-quantitative methods of acid-base analysis during haemorrhage followed by fluid resuscitation.

Study design Randomised control crossover study.

Animals Six domestic cats (mean ±SD: 21 ±1 months old; 4.9 ±1.2 kg).

Methods Venous blood samples were taken during a health check, before haemorrhage, after haemorrhage and then at 30-minute intervals during fluid resuscitation, and again a day later. The cats were anesthetised (buprenorphine, alfaxalone, isoflurane in oxygen, fixed end-tidal concentration of 1.7%) and underwent the following treatments: 1) control, with mild haemorrhage and no resuscitation; or severe haemorrhage followed by 2) lactated Ringer's solution (LRS); and 3) 6% tetrastarch 130/0.4 (Voluven) for resuscitation. The LRS and Voluven were administered at 60- and 20-ml kg^{-1} $hour^{-1}$, respectively, for 120 minutes. Variables used for the analysis methods were measured or calculated from the blood samples and then compared using a general linear mixed model.

Results The total blood loss was 10.2 ±2.3, 29.3 ±9.0 and 29.1 ±6.3 mL kg^{-1} for control, LRS and Voluven, respectively. The total volume of LRS and Voluven administered was 120 and 40 mL kg^{-1}, respectively. All cats were acidaemic during anaesthesia regardless of the treatment. The blood pH decreased from the pre-treatment value of 7.35 (± 0.06) to 7.25 (± 0.06). The pH rose to 7.32 (± 0.08) during the resuscitation phase in all treatments at 120 minutes (time: $p < 0001$). The change in variables within each method were able to detect acid-base derangements in all treatments and could explain the origin of the acidaemia.

Conclusion and clinical relevance The pH was similar among treatments, although differences in the variables used to interpret acid-base status were found between control and the two treatments where haemorrhage was followed by resuscitation. The semi-quantitative method was subjectively more informative compared to the other methods, but its clinical advantage is unclear.

4.2 Introduction

The blood acid-base status of animals is regulated to maintain the pH within a narrow range, which in healthy cats is 7.34 to 7.43 (Hopper et al. 2014a). The essence of this tight regulation of pH range is to ensure optimal enzyme activity that is required for metabolism and regulation of organ function. Enzyme functional activity decreases or halts when the blood or tissues undergo extreme acid-base changes, leading to altered organ function and potentially failure (Mitchell et al. 1972; Crimi et al. 2012). Acidaemia has been reported in 49% of all dogs and cats that were admitted to one teaching hospital and underwent acid-base analysis (Hopper & Epstein 2012). In humans, acidaemia alters cardiovascular system function by causing a decrease in systemic vascular resistance and cardiac output (Crimi et al. 2012). Also, in acidaemic humans, the oxygen-binding capacity of haemoglobin is decreased (Mitchell et al. 1972). The decrease in organ and system function in dogs and cats during acidaemia are thought to be similar to humans and could contribute to mortality (Hopper & Epstein 2012). Reports of acid-base balance in conscious healthy cats emerged in the literature from the 1960s and still appears to be sporadic (Fink & Schoolman 1963, Herbert & Mitchell 1971; Tamura et al. 2015). The routine measurement of acid-base status, especially in emergency and critical care of ill or trauma cats is becoming increasingly relevant to modern case management (Elliott et al. 2003; Lee et al. 2003; Drobatz & Cole 2008; Hopper & Epstein 2012; Ha et al. 2013; Hopper et al. 2014a, b). However, there are scant reports of acid-base balance in anaesthetised cats (Souza et al. 2005); and even less describing acid-base status in cats undergoing severe haemorrhage and fluid resuscitation.

Severe haemorrhage, in veterinary species, is defined as a blood loss of 40% or more and if the blood is lost acutely then compensatory mechanisms may fail and signs of haemorrhagic shock develop (Jutkowitz 2004). In cats, the blood volume ranges from 52.6 ± 6.8 to 59.6 ± 5.8 mL kg^{-1} (Groom et al. 1965; Mott 1968). Therefore, severe haemorrhage, in this study, was defined as a volume of blood loss exceeding 22 mL kg^{-1} of blood was lost. Mild haemorrhage is considered to be a volume of blood loss that does not require replacement and < 10 mL kg^{-1} (Jutkowitz 2004) Resuscitation fluids are administered at ratios relative to the volume of blood being lost. To investigate the effects of volume replacement and fluid tolerance of cats a 4:1 ratio for the lactated Ringer's solution and a 1.5:1 ratio for the tetrastarch 6% 130/0.4 was used. The ratio dose rates were similar to the recommended shock dose fluid rates (Broadstone 1999; Rozanski & Rondeau 2002).

Understanding changes in acid-base status in cats is complicated by the use of different methods to assess acid-base status. Berend (2013) surmises that understanding the pathophysiology of acid-base in human medicine is confusing, irrational and controversial because there are many methods that can be used to analyse acid-base status with no standardised approach. Fortunately, in veterinary medicine, there have only been few reports detailing different methods of evaluating acid-base status decreasing the level of confusion. These methods are the 1) Henderson-Hasselbalch, 2) Stewart, and 3) the semi-quantitative (Hopper et al. 2014a, b).

The methods used in evaluation of acid-base status rely on the measurement or calculation of variables; and these variables are expected to change during anaesthesia, haemorrhage and fluid resuscitation (Hopper et al. 2014a, b; Muir 2017). The present study aimed to compare the variables used in the Henderson-Hasselbalch, Stewart and semi-quantitative methods of acid-base analysis during severe haemorrhage followed by two different resuscitation treatments and a control treatment.

4.3 Materials and Methods

4.3.1 Animals, study design and treatments

Six neutered domestic cats (three males and three females; 21 ± 1 months old; 4.9 ± 1.2 kg) from a research colony housed in a communal indoor-outdoor cattery were used. The study was approved by the animal ethics committees of the Universities of Pretoria (v006-15) and Witwatersrand (2017-10-68-C-AREC) before data collection commenced. This study was part of a larger fluid resuscitation project and only data relevant to the present study are reported.

The cats were allocated randomly (balanced single block design; www.randomization.com) to undergo three treatments, at 2 month intervals. The treatments were divided into two phases, the haemorrhage phase and resuscitation phase, as follows:

Treatment control: Cats underwent a waiting period of 15 minutes to simulate purposeful haemorrhage followed by a sham resuscitation phase of 120 minutes.

Treatment LRS: Cats underwent a purposeful haemorrhage followed by an isotonic crystalloid (lactated Ringer's solution; Fresenius Kabi; RSA) infusion at 60 ml kg^{-1} $hour^{-1}$ for 120 minutes.

Treatment Voluven: Cats underwent a purposeful haemorrhage followed by a 6% tetrastarch 130/0.4 suspended in 0.9% saline (Voluven; Fresenius Kabi; RSA) infusion at 20 ml kg^{-1} $hour^{-1}$ for 120 minutes.

The endpoint for the purposeful haemorrhage was either a 1) maximum blood withdrawal of 30 ml kg^{-1}; or 2) mean arterial blood pressure of < 48 mmHg that persisted for at least 3 minutes.

4.3.2 Study procedures

A health check through clinical examination including blood collection from the jugular vein was done one week prior to treatments. None of the cats were excluded from the study following the pre-treatment evaluations. Food, but not water, was withheld eight hours before treatment. On the morning of treatment, the cat was premedicated with buprenorphine hydrochloride (0.02 mg kg^{-1}; Temgesic 0.3 mg ml^{-1}; Reckitt Benckiser Healthcare; RSA) intramuscularly and left undisturbed for 45 minutes. A catheter (22 gauge; Jelco; Smith Medical International; UK) was aseptically inserted into one of the cephalic veins and secured. Alfaxalone (Alfaxan-CD RTU 10mg ml^{-1}; Afrivet; RSA) was administered intravenously to induce anaesthesia. The trachea was intubated with a cuffed endotracheal tube, which was secured in place and connected to a paediatric circle breathing system (15 mm internal diameter; Compact paediatric breathing system; Intersurgical; RSA). General anaesthesia was maintained by delivering isoflurane (Isofor; Safeline Pharmaceuticals; RSA) in oxygen at a fixed flow rate of 80 ml kg^{-1} $minute^{-1}$. The vaporiser initially set at 2% to achieve a target end-tidal isoflurane concentration (FE′Iso) of 1.7% which was standardised for the study and maintained throughout the procedures (Shaughnessy & Hofmeister 2014). The ventral neck region was clipped and surgically scrubbed before the cat was transferred to the procedure table and positioned in dorsal recumbency. An isotonic crystalloid fluid (Lactated Ringer's solution; Fresenius Kabi, RSA) was infused by an electronic device (Infusomat Space; B-Braun; RSA) at 5 ml kg^{-1} $hour^{-1}$ via a y-ported administration set connected to the cephalic catheter for peri-anaesthetic maintenance fluids (maintenance fluids).

Monitoring of physiological parameters was achieved by placing probes and leads and connecting them to a multiparameter monitor (Datex-Ohmeda Cardiocap 5; Datex; Finland), as follows: heart rate by electrocardiogram, peripheral oxygen haemoglobin saturation by pulse oximetry, end-tidal carbon dioxide and respiratory rate by capnography, and oesophageal temperature. A catheter (22 Gauge, 50 mm, Arrow arterial catheterization set; Arrow International; PA, USA) was inserted into the right pre-superficialised (superficialised 15 months earlier during gonadectomy) carotid artery by cut-down technique to allow for invasive blood pressure monitoring and intermittent blood sampling for gas analysis. A catheter (22 Gauge, 50 mm, Arrow arterial catheterization set; Arrow International; PA, USA) was inserted percutaneously into the left jugular vein for intermittent blood sampling and facilitation of purposeful haemorrhage later. Both catheters were inserted using the Seldinger technique (Seldinger 1953).

Once instrumentation was completed, then one of the three randomly allocated treatments commenced. During the purposeful haemorrhage phase blood was withdrawn manually into a semi-closed system using 20 ml syringes primed with citrate-phosphate-dextrose (4 mL; JMS blood bag, 450 mL; JMS Singapore PTE LTD; Singapore) via the jugular catheter at a targeted rate of 2 ml kg^{-1} $minute^{-1}$ until reaching one of two endpoints. During the resuscitation phase, the randomised resuscitation fluid was infused via a second electronic device (Infusomat Space; B-Braun; RSA), where its administration set was connected to the y-port of the maintenance fluid administration set. Both fluids were administered simultaneously. Blood samples were obtained and physiological variables recorded at fixed time-points during the haemorrhage and resuscitation phase.

On completion of the study procedures the monitoring equipment and catheters, except the jugular catheter, were removed and the cat was then transferred to the intensive care unit of the hospital for recovery and overnight observation. The carotid artery catheter was removed and digital pressure was applied until a stable clot was formed. If a stable clot did not form within 15 minutes then a haemostatic absorbable mesh (BloodStop iX; Life Science Plus; USA, CA) was applied using digital pressure for 10 minutes, or until haemostasis was achieved. Then the cut-down incision site was sutured using a two-layer closure technique with absorbable suture material (MonoPlus 5/0; B Braun). The cat received a single subcutaneous injection of meloxicam (0.2 mg kg^{-1}; 5 mg mL^{-1} Petcam; CiplaVet; RSA) and intravenous injection of buprenorphine (0.03 mg kg^{-1}). The next day, blood was sampled and the jugular catheter was removed before the cat was transferred back to the cattery. The cats were monitored for

two days after the procedures by daily clinical examinations and observation of their social, drinking and eating behaviours. All cats were adopted and rehomed one month after conclusion of the study.

4.3.3 Data collection and analysis

The total blood loss at each time point was cumulative and included the controlled haemorrhage volume (not applicable in control) and all blood samples for profiling. The blood loss in control was defined as mild haemorrhage (<10 ml kg^{-1}). A severe haemorrhage was defined when more than 22 mL kg^{-1} (> 40% assumed circulating volume) of blood was lost. The total volumes of fluids that were administered during resuscitation were used for reporting because all cats were administered maintenance fluids during all treatments and that volume (10 mL kg^{-1} over 120 minutes) was considered negligible. Data relevant to the present study were collected in the form of measuring or calculating of variables used in the three acid-base analysis methods during the health check, treatment period (before haemorrhage phase [-15], immediately after haemorrhage phase [0], at 30 minute intervals during the resuscitation phase until 120 minutes [30, 60, 90, 120]); and a day later (24 hours).

Venous blood samples were drawn from the jugular vein using a needle and syringe and decanted into serum vacuum storage tubes (serum BD Vacutainer tube, Becton Dickinson and Company; UK) during the health check; or via the jugular catheter into a syringe and immediately decanted into serum vacuum storage tubes during the treatment period and a day later. The first 1.5 mL of blood was always discarded prior to drawing the actual blood samples via the jugular catheter. The blood samples were submitted immediately to the onsite laboratory for analyses of serum biochemistry (serum albumin, lactate) and electrolyte (sodium, chloride, potassium, phosphate, ionized calcium) concentrations using daily-calibrated machines by experienced veterinary clinical pathologists. The venous blood samples for acid-base determination were collected into heparinized syringes (BD A-line; Becton Dickinson and Company; UK) and analysed within 10 minutes, using a daily-calibrated benchtop blood gas analyser (interpreted at a fixed temperature of 37°C; RapidPoint 500 System; Siemens; South Africa).

The blood gas analyser measured the pH and venous partial pressure of carbon dioxide ($PvCO_2$) by a potentiometric method using standard ionselective electrode (ISE) technology and modified

potentiometric method based on the principles of the Severinghaus electrode, respectively; and calculated the actual and standard bicarbonate ion concentration [HCO_3^-(act) and HCO_3^-(std)] and extra cellular fluid and blood base excess [BE(ecf) and BE(B)] using the various equations according to Clinical Laboratory Standards Institute C46-A2 (RapidPoint 500 Operator's Manual; Siemens). The measured variables were used in the formulas to evaluate acid-base status (**Table 4.1**).

The data were tested for normality by plotting histograms, evaluating descriptive statistics and performing the Anderson-Darling test for normality. Data were normally distributed and described using mean (± standard deviation). All variables were compared among treatments and over time using a general linear mixed model and significant findings underwent post-hoc pairwise comparisons using Bonferroni correction. Model fits were assessed by visually inspecting residual plots to assess linearity, homogeneity of variances, normality, and outliers. Frequently reported criteria of three methods of acid-base status evaluation were used to interpret the acid-base status in the cats (**Table 4.2**; Hopper et al. 2014a). The acid-base statuses were described among the three methods of acid-base analysis with the aid of tables and figures. The cumulative blood loss was calculated by adding the purposeful haemorrhage volume and the serial blood sampling volumes over time for each cat per treatment and converted to a mL kg^{-1} value. Data were compared using commercially available software (MiniTab 18.1; Minitab Inc., PA, USA) and significance interpreted at $p < 0.05$.

Table 4.1 Equations used to calculate various variables required to interpret acid-base status using the Henderson-Hasselbalch, Stewart and semi-quantitative methods of analysis.

Variable	Equations
Anion gap	$([Na^+] + [K^+]) - ([HCO_3^-(act)] + [Cl^-])$
SIDapparent	$([Na^+] + [K^+] + [Ca^{2+}]) - [Cl^-]$
Albumin contribution	Measured albumin × ((0.123 × pH) − 0.631) × 10
Phosphorus contribution	Measured phosphorus × 0.323 × ((0.309 × pH) − 0.469)
Atot	Albumin contribution + Phosphorus contribution
SIDeffective	$[HCO_3^-act)]$ + albumin contribution + phosphate contribution
Strong ion gap	SIDapparent − SIDeffective
Free water effect	$0.22([Na^+] - \text{mid-normal } [Na^+])$
Corrected chloride	Measured $[Cl^-]$ × (mid-normal $[Na^+]$/measured $[Na^+]$)
Chloride effect	Mid-normal $[Cl^-]$ − corrected $[Cl^-]$
Phosphate effect	0.58 (Mid-normal [phosphate] − measured [phosphate])
Albumin effect	3.7 (Mid-normal albumin − measured [albumin])
Lactate effect	−1 × [lactate]
Sum of effects	Free water effect + chloride effect + phosphate effect + albumin effect + lactate effect
Unmeasured ions effect	Base excess − sum of effects
Blood gas machine equations	
HCO_3^-(act)	pH + log ($PvCO_2$ x 0.0307) – 6.105
HCO_3^-(std)	$24.5 + 0.9A + (A - 2.9)^2$ x (2.65 + 0.31 x Hb)/1000
	Where: A = BE(B) – (0.2 x Hb x (100-SvO_2))/100
BE(ecf)	HCO_3^-(act) – 24.8 + 16.2(pH – 7.40)
BE(B)	(1 – 0.014 x Hb)[(HCO_3^-(act) – 24.8) + (1.43 x Hb + 7.7)(pH – 7.40)]

Note: Mid-normal values were determined as the central value of the variable calculated from pooled values obtained during the health checks. Albumin and Hb measured in g dL^{-1}; phosphate measured in mg dL^{-1}; electrolytes and lactate measured in mmol L^{-1}

Na^+: sodium concentration; K^+: potassium concentration; HCO_3^-(act): actual bicarbonate ion concertation; Cl^-: chloride concentration; g dL^{-1}: grams per decilitre; mg dL^{-1}: milligrams per decilitre; mmol L^{-1}: millimole per litre; SID, strong ion difference; Atot, total quantity of weak acids; HCO_3^-(std): standard bicarbonate ion concentration; BE(ecf): base excess of the extracellular fluid; BE(B): base excess of the blood; Hb: haemoglobin concentration.

Table 4.2 Criteria used to interpret acid-base status based on the value of variables required for the Henderson-Hasselbalch, Stewart and semi-quantitative methods of analysis.

Analysis variable	Interpretation	Criteria
Traditional acid-base analysis method (Henderson-Hasselbalch)		
Simple disturbances	Metabolic acidosis	pH < 7.34, HCO^{3-} < 18 mmol L^{-1}
	Metabolic alkalosis	pH > 7.43, HCO^{3-} > 26 mmol L^{-1}
	Respiratory acidosis	pH < 7.34, $PvCO_2$ > 39 mmHg
	Respiratory alkalosis	pH > 7.43, $PvCO_2$ < 34 mmHg
Mixed disturbances	Abnormalities present for both $PvCO_2$ and HCO^{3-} then the cats were reported to have two co-existing abnormalities	
Metabolic acidosis further classified by anion gap (AG)	Metabolic acidosis associated with increased AG	AG > 20 mmol L^{-1}
	Metabolic acidosis not associated with increased	AG < 20 mmol L^{-1}
Stewart acid-base analysis method		
Apparent strong ion difference (SIDa)	Increased SIDa metabolic alkalosis	SIDa > 44 mmol L^{-1}
	Decreased SIDa metabolic acidosis	SIDa < 40 mmol L^{-1}
Total weak acids (Atot)	Increased Atot metabolic acidosis	Atot > 17 mmol L^{-1}
	Decreased Atot metabolic alkalosis	Atot < 8 mmol L^{-1}
Unmeasured anions	Increased SIG	SIG > 9 mmol L^{-1}
Semi-quantitative acid-base analysis method		
Free water effect	Dilutional acidosis	Free water effect < −1.0 mmol L^{-1}
	Contraction alkalosis	Free water effect > 0.7 mmol L^{-1}
Chloride effect	Acidosis	Chloride effect < −4.0 mmol L^{-1}
	Alkalosis	Chloride effect > 5.0 mmol L^{-1}
Phosphorus effect	Acidosis	Phosphorus effect < −1.0 mmol L^{-1}
	Alkalosis	Phosphorus effect > 1.0 mmol L^{-1}
Albumin effect	Acidosis	Albumin effect < −3.0 mmol L^{-1}
	Alkalosis	Albumin effect > 3.0 mmol L^{-1}
Lactate effect	Acidosis	Lactate effect > −2.0 mmol L^{-1}
Unmeasured ions effect	Unmeasured acids	Unmeasured ions effect < −0.5 mmol L^{-1}
	Unmeasured alkalis	Unmeasured ions effect > 0.5 mmol L^{-1}

HCO^{3-}: bicarbonate ion concentration; $PvCO_2$: venous partial pressure of carbon dioxide; mmol L^{-1}: millimole per litre; mmHg: millimetres mercury.

4.4 Results

The cumulative blood loss at time 0 was 3.4 ±0.8, 22.3 ±7.7 and 21.6 ±4.7 mL kg^{-1} for control, LRS and Voluven, respectively; at time 120 the blood loss was 10.2 ±2.3, 29.3 ±9.0 and 29.1 ±6.3 mL kg^{-1} (**Table 4.3**). The total volume of LRS and Voluven that were administered at time 120 minutes were 120 and 40 mL kg^{-1}, respectively. The haemoglobin concentration decreased in all treatments during general anaesthesia and the concentration dropped even further in LRS and Voluven from 30 to 120 minutes (treatment: $p < 0.001$; time: $p < 0.001$; treatment x time: $p < 0.001$).

All cats were acidaemic during anaesthesia regardless of the treatment with the pooled averaged venous blood pH values decreasing significantly from the pre-treatment value of 7.35 (± 0.06) to 7.25 (± 0.06) before the haemorrhage phase. The haemorrhage phase did not alter the pH, which was 7.25 (± 0.06) at the end of this phase, but it increased steadily during the resuscitation phase, even in the control, to 7.32 (± 0.08) at 120 minutes. The pH trends over time among the treatments are presented in **Figure 4.1** and **Table 4.3** (time: $p < 0.001$).

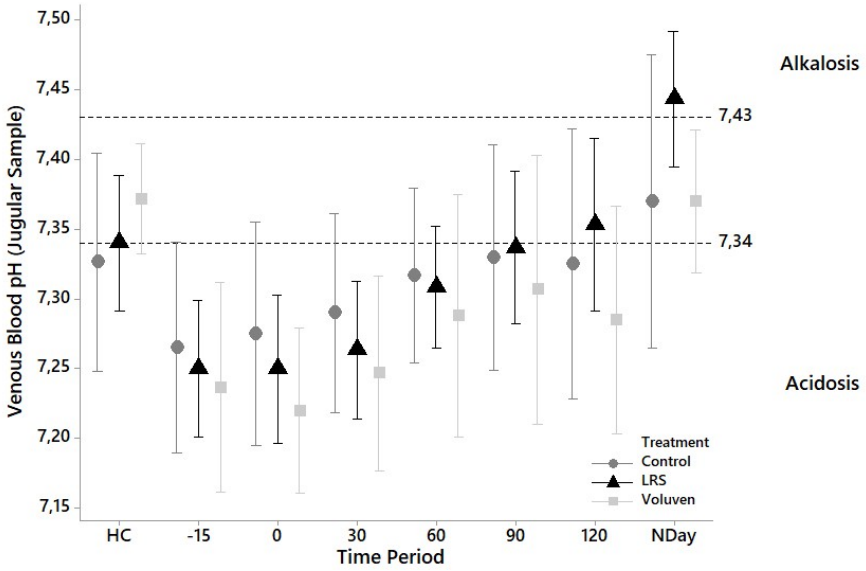


Figure 4.1 The venous blood pH obtained from a jugular vein in cats undergoing three treatments while under isoflurane in oxygen anaesthesia. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. Blood samples were taken during a health check (HC), before haemorrhage (-15), after haemorrhage (0) and then at 30-minute intervals (30, 60, 90, 120) during fluid resuscitation and the next day (ND). Dash lines indicate the reference interval.

Table 4.3 The venous blood pH, variables measured or calculated used in acid-base analysis and cumulative blood loss and resuscitation fluid volumes in isoflurane in oxygen anaesthetised cats undergoing three treatments. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. Blood samples were taken during a health check (HC), before haemorrhage (-15), after haemorrhage (0) and then at 30-minute intervals (30, 60, 90, 120) during fluid resuscitation and the next day (ND).

Parameter	Unit	Treatment	Time Period															
			HC		-15		0		30		60		90		120		NDay	
			x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD
pH		Control	**7.33**	0.07	**7.27***	0.07	**7.28***	0.08	**7.29***	0.07	**7.32**	0.06	**7.33**	0.08	**7.33**	0.09	7.37	0,10
		LRS	7.34	0.05	**7.25***	0.05	**7.25***	0.05	**7.26***	0.05	**7.31***	0.04	7.34	0.05	7.35	0.06	**7.44***	0.05
		Voluven	7.37	0.04	**7.24***	0.07	**7.22***	0.06	**7.25***	0.07	**7.29***	0.08	**7.31***	0.09	**7.29***	0.08	7.37	0.05
Measured and calculated variables required for the three methods of acid-base analysis																		
Na^+	mmol L^{-1}	Control	146	9	139*	8	137*	6	137*	5	136*	4	136*	5	135*	5	152	3
		LRS	148	6	139*	3	138*	6	135*	8	134*	5	132*	4	130*	3	152	2
		Voluven	147	9	138*	4	137*	9	136*	6	137*	6	135*	3	135*	7	150	5
K^+	mmol L^{-1}	Control	3.4	0.4	3.5	0.4	3.6	0.4	3.8*	0.4	3.9*	0.6	3.9*	0.5	3.8*	0.4	4.1	0.6
		LRS	3.3	0.3	3.6	0.2	3.9*	0.3	3.8*	0.3	3.9*	0.3	3.8*	0.3	3.8*	0.3	3.6	0.1
		Voluven	3.5	0.4	3.5	0.2	3.9*	0.3	3.6*	0.2	3.7*	0.3	3.8*	0.4	3.8*	0.6	3.7	0.3
Cl^-	mmol L^{-1}	Control	115	8	113	5	112	3	111	4	111	3	112	3	112	3	120*	2
		LRS	116	3	111	2	111	3	113	5	114	4	112	3	112	3	120*	1
		Voluven	115	7	112	3	113	6	115*†	5	118*†	2	118*†	2	120*†	5	120*	5
Corrected Cl^-	mmol L^{-1}	Control	116	3	119	2	119	2	119	2	121	2	121	2	121	1	116	3
		LRS	115	2	118	3	119	4	124	3	125*	2	126*	2	126*	3	117	1
		Voluven	115	3	119	2	121	3	124	3	127*	4	129*†	2	130*†	3	118*	3
Ca^{2+}	mmol L^{-1}	Control	1.1	0.1	1.1	0.1	1.1	0.1	1.1	0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.2*	0.0
		LRS	1.2*	0.1	1.1	0.1	1.1	0.1	1.1	0.1	1.1	0.1	1.0	0.1	1.0	0.1	1.2*	0.0
		Voluven	1.1	0.1	1.1	0.1	1.1	0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.0	0.1	1.2*	0.1
Phosphate	mg dL^{-1}	Control	4.9	0.6	6.1*	0.6	6.3*	0.6	6.1*	0.6	5.8	0.6	5.6	0.5	5.5	0.7	4.8	0.6
		LRS	4.6	0.4	6.6*	0.5	7.1*†	0.7	5.1*†	1.0	4.9†	0.4	4.5†	0.4	4.3†	0.3	4,3	0.6
		Voluven	4.7	0.4	6.7*	0.4	6.6*	0.5	5.9*	0.2	5.5	0.3	4.9	0.5	4.8	0.6	4.3	0.9
Albumin	g dL^{-1}	Control	3.3	0.5	2.9	0.4	2.8	0.4	2.8†	0.4	2.8†	0.4	2.6†	0.4	2.5†	0.4	3.0	0.8
		LRS	3.2	0.4	2.9	0.4	2.6	0.4	1.6*	0.4	1.5*	0.2	1.4*	0.3	1.4*	0.3	2.9	0.5
		Voluven	3.3	0.5	2.8	0.4	2.4	0.5	1.7*	0.3	1.4*	0.3	1.3*	0.2	1.1*	0.2	3.0	0.5
Hb	g dL^{-1}	Control	13.6	2.1	8.6*	0.6	8.4*	0.7	8.6*	0.6	9.3*	1.2	8.6*	0.8	8.8*	0.6	10.6	1.5
		LRS	13.1	0.7	8.9*	0.6	9.8*	1.0	5.3*†	0.9	4.6*†	0.6	4.4*†	0.7	4.6*†	1.2	8.5*†	1.4
		Voluven	13.4	1.4	8.5*	1.1	9.4*	1.4	5.7*†	1.2	4.3*†	0.6	3.8*†	0.6	3.4*†	0.6	8.7*	0.8

Table 4.3 continued

Parameter	Unit	Treatment	Time Period															
			HC		-15		0		30		60		90		120		NDay	
			x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD
Lactate	mmol L^{-1}	Control	1.4	0.6	1.4	0.3	1.5	0.3	1.4	0.4	1.7	0.5	1.8	0.3	1.9	0.3	3.3	1.8
		LRS	1.6	0.4	1.6	0.3	1.9	0.7	3.4†	1.4	3.4†	1.8	3.9†	1.7	3.4†	0.5	2.0	0.5
		Voluven	1.7	0.6	1.6	0.6	2.2	1.3	1.4	0.3	1.1	0.3	1.4	0.8	1.3	0.3	2.8	0.8
Cumulative blood loss volumes during haemorrhage phase (purposeful haemorrhage and blood sampling) and resuscitation phase (blood sampling)																		
Blood loss	mL kg^{-1}	Control	-	-	1.3	0.3	3.4	0.8	4.7	1.1	6.8	1.6	8.1	1.9	10.2	2.3	-	-
		LRS	-	-	1.3	0.3	22.3	7.3	23.6	7.6	25.8	8.1	27.1	8.5	29.3	9.0	-	-
		Voluven	-	-	1.3	0.3	21.6	4.7	23.4	5.2	25.6	5.6	26.9	5.9	29.1	6.3	-	-
Cumulative volume of resuscitation fluid administered during resuscitation phase																		
Fluid volume	mL kg^{-1}	Control	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-
		LRS	0	-	0	-	0	-	30	-	60	-	90	-	120	-	0	-
		Voluven	0	-	0	-	0	-	10	-	20	-	30	-	40	-	0	-

Bolded values are outside the reference interval used in this study. *: significant values over time within a treatment; †: significant value among treatments. Significant values were $p < 0.05$.

x̄: mean; ±SD: standard deviation; Na^+: sodium concentration; mmol L^{-1}: millimole per litre; K^+: potassium concentration; Cl^-: chloride concentration; Ca^{2+}: ionized calcium concentration; Hb: haemoglobin concentration; mg dL^{-1}: milligrams per decilitre; g dL^{-1}: grams per decilitre; ml kg^{-1}: millilitres per kilogram.

All three acid-base status interpretation methods were able to diagnose acid-base disturbances that could explain the overall acidaemia during the general anaesthetic period. A mixed acid-base disturbance was diagnosed during anaesthesia using the traditional Henderson-Hasselbalch method of interpretation. The $PvCO_2$ was above 39 mmHg for the entire duration of general anaesthesia for cats receiving LRS and Voluven, but in cats in the control it returned to values between 34 and 39 mmHg by 30 minutes (**Figure 4.2** and **Table 4.4**; time: $p < 0.001$). Therefore, there was a persistent respiratory acidosis in the LRS and Voluven treatments, but only a transient respiratory acidosis in the control. The HCO_3^-(act) was consistently between 18 and 26 mmol L^{-1} during all time periods, which ruled out a metabolic acidosis (treatment: $p = 0.001$). However, the BE(ecf) was consistently more negative than -5 mmol L^{-1}, which indicated a metabolic acidosis, except for the LRS treatment in which the BE(ecf) was above -5 mmol L^{-1} at 120 minutes and a day after treatments (treatment: $p < 0.001$; time: $p < 0.001$). The HCO_3^-(std) (treatment: $p = 0.003$; time: $p < 0.001$) and BE(B) (treatment: $p < 0.001$; time: $p < 0.001$) had similar trends over time to the HCO_3^-(act) and BE(ecf), respectively. The anion gap was never above 20 mmol L^{-1} for any treatment over time (treatment: $p < 0.001$; time: $p < 0.001$; treatment x time: $p < 0.001$).

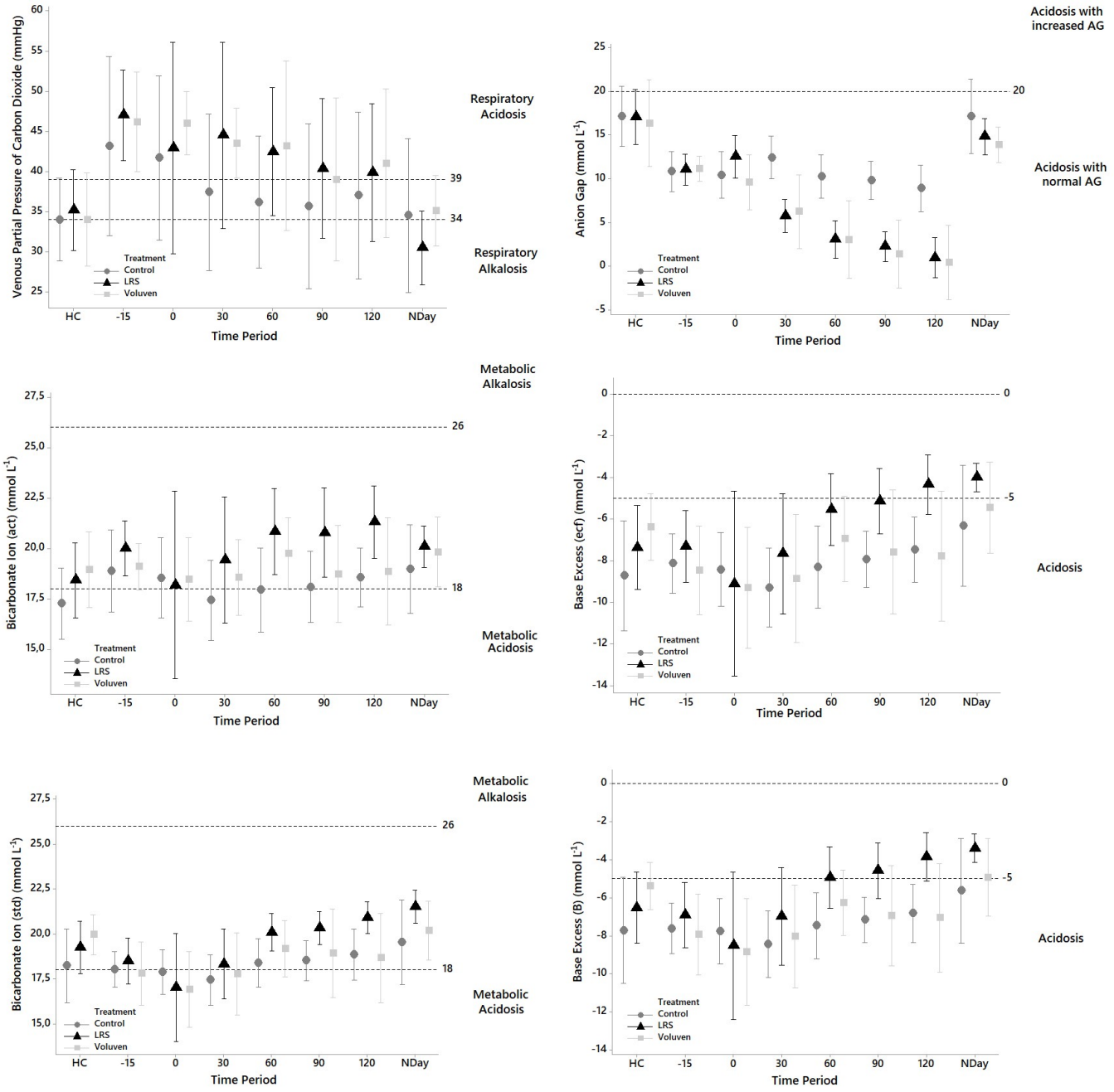


Figure 4.2 The variables used to interpret blood pH using the Henderson-Hasselbalch method, anion gap and base excess in cats undergoing three treatments while under isoflurane in oxygen anaesthesia. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. Blood samples were taken during a health check (HC), before haemorrhage (-15), after haemorrhage (0) and then at 30-minute intervals (30, 60, 90, 120) during fluid resuscitation and the next day (ND). Dash lines indicate the reference interval.

Table 4.4 The variables used in the Henderson-Hasselbalch and traditional methods of acid-base analysis in isoflurane in oxygen anaesthetised cats undergoing three treatments. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. Blood samples were taken during a health check (HC), before haemorrhage (-15), after haemorrhage (0) and then at 30-minute intervals (30, 60, 90, 120) during fluid resuscitation and the next day (ND).

Parameter	Unit	Treatment	Time Period															
			HC		-15		0		30		60		90		120		NDay	
			x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD
$PvCO_2$	mmHg	Control	34	5	**43***	11	**42***	10	37	9	36	8	36	10	37	10	35	9
		LRS	35	5	**47***	5	**43***	13	**45***	11	**42***	8	**40***	8	**40***	8	**31**	4
		Voluven	34	6	**46***	6	**46***	4	**44***	4	**43***	10	39*	10	**41***	9	35	4
HCO^{3-}(act)	mmol L^{-1}	Control	**17.3**	1.7	18.9	1.7	18.5	1.9	**17.4**	1.9	**17.9**	2.0	18.1	1.7	18.6	1.4	19.0	2.1
		LRS	18.4	1.8	20.0	1.3	18.2	4.4	19.4†	3.0	20.8†	2.0	20.8†	2.1	21.3†	1.7	20.1	1.0
		Voluven	18.9	1.8	19.1	1.1	18.5	2.0	18.6	1.8	19.7	1.7	18.7	2.3	18.6	2.5	19.8	1.7
HCO_3^-(std)	mmol L^{-1}	Control	18.2	1.9	18.0	0.9	**17.9**	0.9	**17.5**	1.3	18.4	1.3	18.5	1.1	18.9	1.4	19.5	2.2
		LRS	19.3	1.4	18.5*	1.2	**17.0***†	2.9	18.3*	1.8	20.1	1.0	20.3	0.9	20.9†	0.8	21.5	0.9
		Voluven	20.0	1.1	**17.8***†	1.7	**16.9***†	2.0	**17.8***	2.2	19.2	1.5	18.9	2.4	18.7	2.4	20.2	1.6
BE(ecf)	mmol L^{-1}	Control	**-8.7**	2.5	**-8.1**	1.4	**-8.4**	1.7	**-9.3***	1.8	**-8.3**	1.9	**-7.9**	1.3	**-7.5**	1.5	**-6.3***	2.8
		LRS	**-7.4**	1.9	**-7.3**	1.6	**-9.1***	4.2	**-7.7**†	2.8	**-5.6***†	1.6	**-5.2***†	1.5	-4.4*†	1.4	-4.0*†	0.7
		Voluven	**-6.4**	1.5	**-8.5***	2.0	**-9.3***	2.8	**-8.9***	2.9	**-7.0***	1.9	**-7.6***	2.8	**-7.8***	3.0	**-5.5**	2.1
BE(B)	mmol L^{-1}	Control	**-7.7**	2,7	**-7.6**	1.3	**-7.7**	1.6	**-8.4**	1.7	**-7.5**	1.7	**-7.2**	1.1	**-6.8**	1.5	**-5.6***	2.6
		LRS	**-6.5**	1.8	**-6.9**	1.6	**-8.5***	3.7	**-7.0***†	2.5	-4.9†	1.5	-4.6†	1.4	-3.8†	1.2	-3.4†	0.7
		Voluven	**-5.4**	1.2	**-7.9***	2.0	**-8.8***	2.7	**-8.0***	2.7	**-6.3**	1.6	**-6.9***	2.5	**-7.1***	2.7	-4.9	2.0
Anion Gap	mmol L^{-1}	Control	17.2	3.3	10.8*	2.2	10.4*	2.6	12.4*	2.3	10.3*	2.4	9.8*	2.1	8.9*	2.5	17.2	4.0
		LRS	17.1	3.0	11.1*	1.7	12.5*	2.3	5.7*†	1.8	3.1*†	2.1	2.2*†	1.6	1.0*†	2.2	14.8	2.0
		Voluven	16.4	4.7	11.1*	1.4	9.6*	3.0	6.2*†	4.0	3.0*†	4.2	1.4*†	3.7	0.4*†	4.1	13.9	1.9

Bolded values are outside the reference interval used in this study. *: significant values over time within a treatment; †: significant value among treatments. Significant values were $p < 0.05$.

x̄: mean; ±SD: standard deviation; PvCO2: venous partial pressure of carbon dioxide; mmHg: millimetres mercury; HCO^{3-}(act): actual bicarbonate ion concentration; mmol L^{-1}: millimole per litre; HCO^{3-}(std): standard bicarbonate ion concentration; BE(ecf): base excess of the extracellular fluid; BE(B): base excess of the blood.

The Stewart method diagnosed acidotic and alkalotic metabolic processes. The SIDapparent was consistently under 40 mmol L^{-1} for all treatments over time, thus indicating metabolic acidosis (**Figure 4.3** and **Table 4.5**; treatment: p < 0.001; time: p < 0.001; treatment x time: p = 0.003); however, the Atot was under 8 mmol L^{-1} for LRS and Voluven treatments from 30 to 120 minutes, which indicated metabolic alkalosis, but otherwise the values were within the 8 to 17 mmol L^{-1} range, similar to the control, during the health check and next day (treatment: p < 0.001; time: p < 0.001; treatment x time: p < 0.001). The strong ion gap was normal for all treatments over time (treatment: p < 0.001; time: p < 0.001).

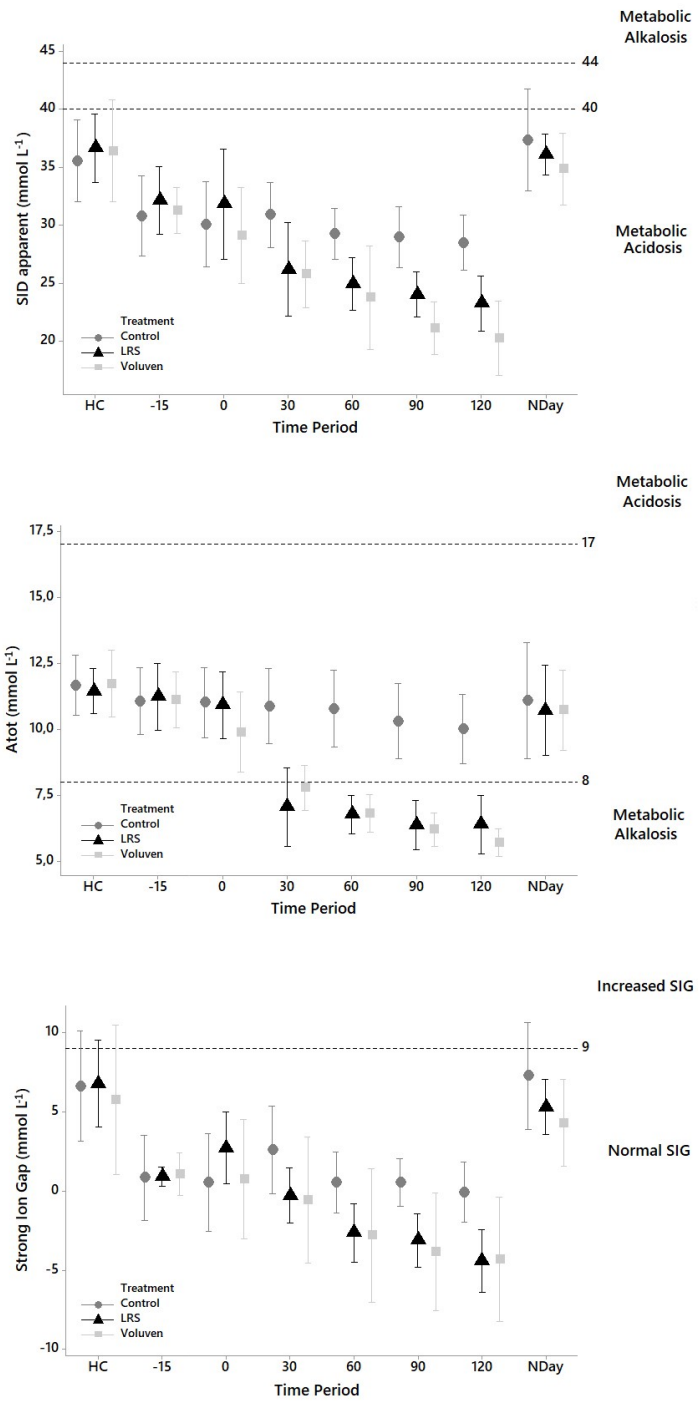


Figure 4.3 The variables used to interpret blood pH using the Stewart method in cats undergoing three treatments while under isoflurane in oxygen anaesthesia. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. Blood samples were taken during a health check (HC), before haemorrhage (-15), after haemorrhage (0) and then at 30-minute intervals (30, 60, 90, 120) during fluid resuscitation and the next day (ND). Dash lines indicate the reference interval.

Table 4.5 The variables used in the Stewart method of acid-base analysis in isoflurane in oxygen anaesthetised cats undergoing three treatments. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. Blood samples were taken during a health check (HC), before haemorrhage (-15), after haemorrhage (0) and then at 30-minute intervals (30, 60, 90, 120) during fluid resuscitation and the next day (ND).

Parameter	Unit	Treatment	Time Period															
			HC		-15		0		30		60		90		120		NDay	
			x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD
SIDa	mmol L^{-1}	Control	**35.6**	3.4	**30.8***	3.3	**30.1***	3.5	**30.9***	2.7	**29.3***	2.1	**29.0***	2.5	**28.5***	2.2	**37.4**	4.2
		LRS	**36.7**	2.8	**32.2***	2.8	**31.8***	4.5	**26.2*†**	3.8	**25.0*†**	2.2	**24.0*†**	1.8	**23.3*†**	2.3	**36.1**	1.7
		Voluven	**36.4**	4.2	**31.3***	1.9	**29.1***	3.9	**25.8*†**	2.7	**23.8*†**	4.2	**21.1*†**	2.2	**20.2*†**	3.0	**34.9**	2.9
Albumin contribution	mmol L^{-1}	Control	8.8	1.4	7.6	1.1	7.4*	1.1	7.4*	1.1	7.5*	1.3	7.1*	1.3	6.9*	1.3	8.3	1.9
		LRS	8.8	1.1	7.5	1.0	6.9*	1.1	4.1*†	1.0	4.0*†	0.6	3.8*†	0.8	3.9*†	1.0	8.2	1.4
		Voluven	9.0	1.2	7.3*	1.2	6.2*	1.3	4.4*†	0.8	3.7*†	0.7	3.4*†	0.6	2.9*†	0.6	8.2	1.3
Phosphorus contribution	mmol L^{-1}	Control	2.8	0.3	3.5*	0.3	3.6*	0.3	3.5*	0.3	3.3*	0.3	3.2	0.3	3.2	0.4	2.8	0.3
		LRS	2.7	0.2	3.8*†	0.3	4.0*†	0.4	2.9	0.5	2.8	0.2	2.6	0.2	2.5	0.2	2.6	0.4
		Voluven	2.8	0.2	3.8*†	0.2	3.7*	0.3	3.4*	0.1	3.1	0.2	2.9	0.3	2.8	0.3	2.5	0.5
Atot	mmol L^{-1}	Control	11.7	1.1	11.1	1.2	11.0	1.3	10.9	1.4	10.8	1.4	10.3	1.4	10.0	1.2	11.1	2.1
		LRS	11.5	0.8	11.2	1.2	10.9	1.2	**7.1*†**	1.4	**6.8*†**	0.7	**6.4*†**	0.9	**6.4*†**	1.1	10.7	1.6
		Voluven	11.7	1.2	11.1	1.0	9.9	1.4	**7.8*†**	0.8	**6.8*†**	0.7	**6.2*†**	0.6	**5.7*†**	0.5	10.7	1.4
SIDe	mmol L^{-1}	Control	28.9	2.4	30.0	2.9	29.6	3.0	28.3*	2.8	28.7*	2.6	28.4*	2.3	28.6*	1.8	30.1	3.0
		LRS	29.9	2.0	31.2	2.4	29.1	5.0	26.5*†	2.3	27.6*	2.3	27.2*	2.2	27.7*	1.9	30.8	2.0
		Voluven	30.7	2.1	30.2	1.9	28.4*	3.0	26.4*†	2.0	26.6*	1.9	24.9*†	2.3	24.6*†	2.4	30.6	2.0
SIG	mmol L^{-1}	Control	6.6	3.3	0.9*	2.6	0.5*	2.9	2.6*	2.7	0.5*	1.8	0.6*	1.4	-0.1*	1.8	7.3	3.2
		LRS	6.8	2.6	0.9*	0.6	2.7*	2.2	-0.3*	1.7	-2.6*†	1.7	-3.1*†	1.6	-4.4*†	1.9	5.3	1.7
		Voluven	5.8	4.5	1.1*	1.3	0.8*	3.6	-0.6*	3.8	-2.8*†	4.0	-3.8*†	3.5	-4.3*†	3.7	4.3	2.6

Bolded values are outside the reference interval used in this study. *: significant values over time within a treatment; †: significant value among treatments. Significant values were $p < 0.05$.

x̄: mean; ±SD: standard deviation; SIDa: apparent strong ion difference; mmol L^{-1}: millimole per litre; Atot: total weak acids; SIDe: effective strong ion difference; SIG: strong ion gap.

The semi-quantitative method diagnosed acidotic and alkalotic metabolic states. The free water effect indicated a dilutional acidosis for all treatments during general anaesthesia (**Figure 4.4** and **Table 4.6**; time: $p < 0.001$); and the chloride effect indicated an acidosis from 30 to 120 minutes of the general anaesthesia in all treatments (treatment: $p < 0.001$; time: $p < 0.001$; treatment x time: $p < 0.001$). The lactate effect was more negative than -2 mmol L^{-1} in the LRS treatment predominantly, from 30 to 120 minutes of general anaesthesia, but in all treatments values were below -2 mmol L^{-1} on the next day (treatment: $p < 0.001$; time: $p = 0.001$; treatment x time: $p < 0.001$). Unmeasured acids were also detected, using the unmeasured ions effect, in most treatments during the health check and early stages of the general anaesthesia until 30 minutes (treatment: $p = 0.004$; time: $p < 0.001$). The phosphate effect only detected an acidosis during -15 and 0 minutes of general anaesthesia in cats receiving LRS and Voluven, but this effect was before the resuscitation phase (treatment: $p = 0.001$; time: $p < 0.001$; treatment x time: $p = 0.001$). A metabolic alkalosis was diagnosed by the albumin effect for LRS and Voluven from 30 to 120 minutes during general anaesthesia (treatment: $p < 0.001$; time: $p < 0.001$; treatment x time: $p < 0.001$); and by the unmeasured ions effect that indicated the presence of unmeasured alkalis from 30 to 120 minutes in LRS and Voluven, but not control.

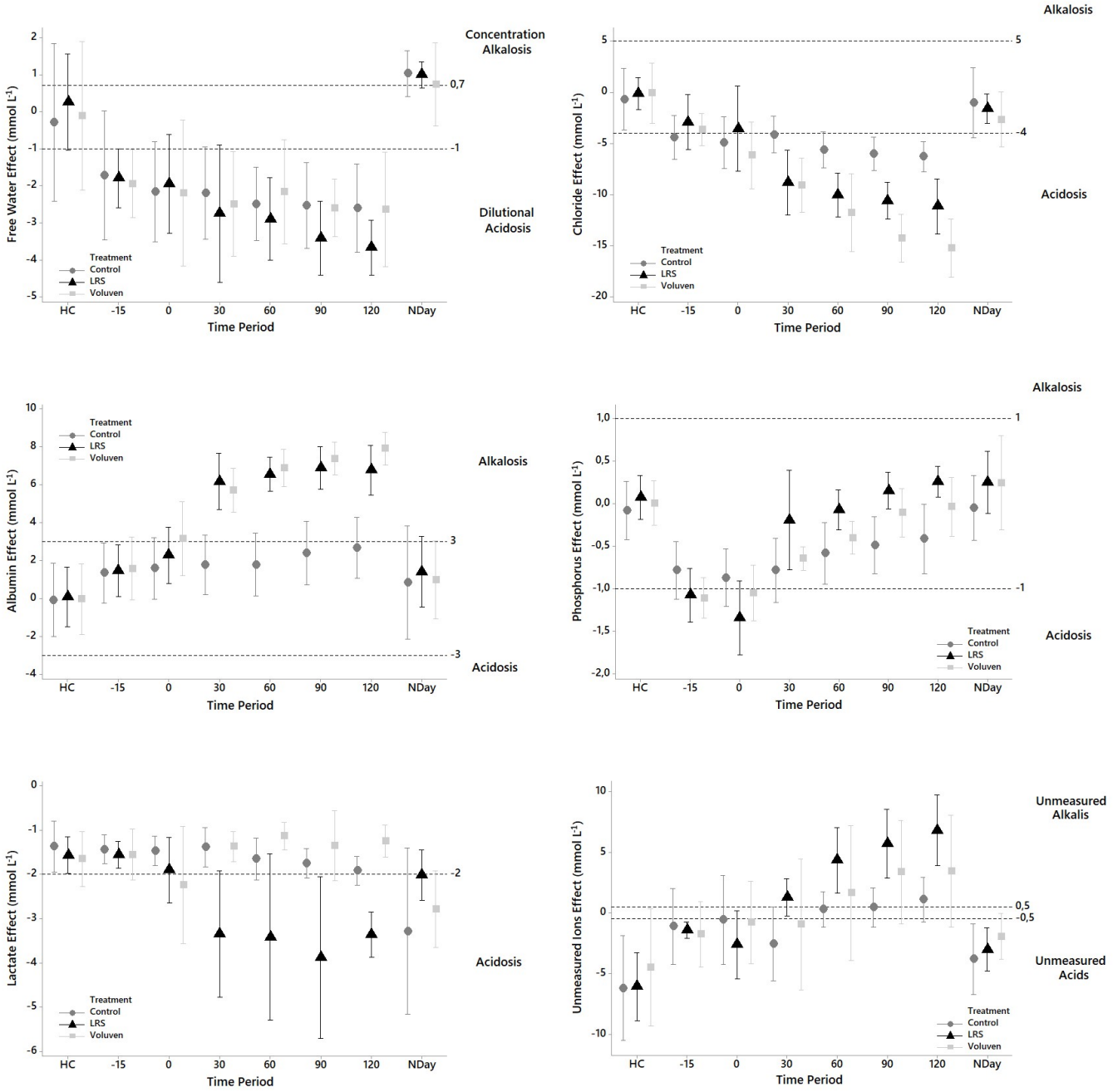


Figure 4.4 The variables used to interpret blood pH using the semi-quantitative method in cats undergoing three treatments while under isoflurane in oxygen anaesthesia. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. Blood samples were taken during a health check (HC), before haemorrhage (-15), after haemorrhage (0) and then at 30-minute intervals (30, 60, 90, 120) during fluid resuscitation and the next day (ND). Dash lines indicate the reference interval.

Table 4.6 The variables used in the semi-quantitative method of acid-base analysis in isoflurane in oxygen anaesthetised cats undergoing three treatments. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. Blood samples were taken during a health check (HC), before haemorrhage (-15), after haemorrhage (0) and then at 30-minute intervals (30, 60, 90, 120) during fluid resuscitation and the next day (ND).

Parameter	Unit	Treatment	Time Period															
			HC		-15		0		30		60		90		120		NDay	
			x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD	x̄	±SD
Free water effect	mmol L^{-1}	Control	-0.3	2.0	**-1.7***	1.7	**-2.2***	1.3	**-2.2***	1.2	**-2.5***	0.9	**-2.5***	1.1	**-2.6***	1.1	**1.0**	0.6
		LRS	0.3	1.2	**-1.8***	0.8	**-1.9***	1.3	**-2.8***	1.8	**-2.9***	1.1	**-3.4***	1.0	**-3.7***	0.7	**1.0**	0.3
		Voluven	-0.1	1.9	**-1.9***	0.9	**-2.2***	1.9	**-2.5***	1.3	**-2.2***	1.3	**-2.6***	0.7	**-2.6***	1.5	0.7	1.1
Chloride effect	mmol L^{-1}	Control	-0.7	2.9	**-4.4**	2.0	**-4.9**	2.4	**-4.1**	1.7	**-5.7***	1.7	**-6.0***	1.5	**-6.3***	1.4	-1.0	3.3
		LRS	-0.1	1.5	-2.9	2.6	-3.6	4.0	**-8.8*†**	3.0	**-10.1*†**	2.1	**-10.6*†**	1.7	**-11.1*†**	2.6	-1.6	1.4
		Voluven	-0.1	2.8	-3.7	1.5	**-6.2**	3.1	**-9.1*†**	2.5	**-11.8*†**	3.6	**-14.3*†**	2.2	**-15.2*†**	2.7	-2.7	2.5
Phosphorus effect	mmol L^{-1}	Control	-0.1	0.3	-0.8*	0.3	-0.9*	0.3	-0.8*	0.4	-0.6*	0.3	-0.5	0.3	-0.4	0.4	0.0	0.4
		LRS	0.1	0.2	**-1.1***	0.3	**-1.3***	0.4	-0.2*†	0.6	-0.1†	0.2	0.2†	0.2	0.3†	0.2	0.3	0.3
		Voluven	0.0	0.2	**-1.1***	0.2	**-1.0***	0.3	-0.6*	0.1	-0.4*	0.2	-0.1	0.3	0.0	0.3	0.2	0.5
Albumin effect	mmol L^{-1}	Control	-0.1	1.8	1.3	1.5	1.6	1.5	1.8	1.5	1.8	1.6	2.4	1.6	2.7	1.5	0.8	2.8
		LRS	0.1	1.5	1.4	1.3	2.3	1.4	**6.2*†**	1.4	**6.6*†**	0.9	**6.9*†**	1.1	**6.8*†**	1.3	1.4	1.8
		Voluven	0.0	1.8	1.6	1.6	**3.2**	1.9	**5.7*†**	1.1	**6.9*†**	1.0	**7.4*†**	0.8	**7.9*†**	0.8	1.0	2.0
Lactate effect	mmol L^{-1}	Control	-1.4	0.6	-1.4	0.3	-1.5	0.3	-1.4	0.4	-1.7	0.5	-1.8	0.3	-1.9	0.3	**-3.3*†**	1.8
		LRS	-1.6	0.4	-1.6	0.3	-1.9	0.7	**-3.4*†**	1.4	**-3.4*†**	1.8	**-3.9*†**	1.7	**-3.4*†**	0.5	**-2.0**	0.5
		Voluven	-1.7	0.6	-1.6	0.6	**-2.2**	1.3	-1.4	0.3	-1.1	0.3	-1.4	0.8	-1.3	0.3	**-2.8*†**	0.8
Sum of effects	mmol L^{-1}	Control	-2.5	2.9	-7.0*	2.5	-7.8*	3.2	-6.7*	2.1	-8.6*	1.1	-8.4*	1.5	-8.6*	1.6	-2.5	3.2
		LRS	-1.3	1.6	-5.9*	2.0	-6.5*†	3.9	-8.9*	3.9	-9.9*	3.3	-10.8*†	3.4	-11.2*†	3.0	-1.0	1.3
		Voluven	-1.9	3.7	-6.7*	1.3	-8.5*	4.4	-7.9*	2.6	-8.6*	4.3	-10.9*†	2.2	-11.2*†	3.4	-3.5	3.4
Unmeasured ions effect	mmol L^{-1}	Control	**-6.2**	4.1	**-1.1**	3.0	**-0.6**	3.5	**-2.6**	2.9	0.3*	1.4	0.4*	1.5	**1.1***	1.7	**-3.8**	2.8
		LRS	**-6.1**	2.7	**-1.4**	0.6	**-2.6**	2.7	**1.3**	1.5	**4.3*†**	2.6	**5.7*†**	2.7	**6.8*†**	2.8	**-3.0**	1.7
		Voluven	**-4.5**	4.6	**-1.8**	2.6	**-0.8**	3.2	**-1.0**	5.2	**1.6**	5.3	**3.4**	4.0	**3.4**	4.4	**-2.0**	1.8

Bolded values are outside the reference interval used in this study. *: significant values over time within a treatment; †: significant value among treatments. Significant values were $p < 0.05$.

x̄: mean; ±SD: standard deviation; mmol L^{-1}: millimole per litre

4.5 Discussion

The three acid-base status evaluation methods were able to detect simple and complex derangements in all treatments that could explain the origin of the acidaemia. The major variables that changed were PvCO2, HCO_3^-(act) (Henderson-Hasselbalch); SIDapparent, Atot (Stewart); free water effect, chloride effect and albumin effect (semi-quantitative).

The $PvCO_2$ was within normal limits during the control treatment which suggests that the cats were normocapnic. The normocapnia indicated that the depth of anaesthesia was adequate and not causing hypoventilation. The increase in $PvCO_2$ observed during LRS and Voluven treatments could have arisen from of a change in depth of anaesthesia, whereby the low cardiac output enhanced the uptake of the isoflurane making the cats more deeply anaesthetised. This effect has been described in dogs anaesthetised with isoflurane and undergoing haemorrhage-induced hypovolaemia (Mattson et al. 2006). Furthermore, the gap between the arterial and the $PvCO_2$ is usually less than 5 mmHg in anaesthetised or conscious dogs and cats (Dubin et al. 1990). However, during low cardiac output states, the gap tends to widen during the low flow state (Benjamin et al. 1987). This widening gap could also explain the higher $PvCO_2$ in the two treatments where the cats underwent haemorrhage and resuscitation compared to the control treatment.

Both HCO_3^-(act) and HCO_3^-(std) were mostly within published ranges (Hopper et al. 2014a) and did not indicate a metabolic acidosis. The LRS treatment demonstrated an increase in both HCO_3^- over time, which could be explained by lactate that is metabolised into bicarbonate (Gladden 2004). Both calculated BE indicated a metabolic acidosis was present in all treatments, except for LRS at time 90 and 120 minutes and the next day (Hopper et al. 2014a). Both calculations incorporate the HCO_3^-(act) and pH as variables which could explain the rise in both BE for LRS (Russell et al. 1996).

The SIDapparent was below 40 mmol L^{-1} at all time periods, which indicated metabolic acidosis (Hopper et al. 2014a). The acidosis was present mostly during the general anaesthetic period and not during awake states. We made use of frequently reported SIDapparent ranges for interpretation (Hopper et al. 2014a). However, perhaps the acidosis threshold in cats should be decreased to a SIDapparent of 35 mmol L^{-1}; this shift would reflect the measured pH during the health check and following day in our cats during these two time periods when there were theoretically no derangements in acid-base status. Another explanation for the overall low SIDapparent value is that our cats had a

lower sodium ion concentration throughout the study compared to normal published ranges (Hopper et al. 2014a). We speculate that the low sodium ion concentration could be attributed to their diet, water intake and sedentary level of activity within the cattery.

The Atot indicating an alkalinising effect during the resuscitation phase in LRS and Voluven treatments might have been caused by hypoalbuminaemia (Russell et al. 1996). However, the hypoalbuminaemia did not contribute to a difference in the overall pH among treatments and over time in our cats; which is in agreement with the sentiment that the albumin concentration does not influence blood pH (Berend 2013). Despite a similar pH among treatments, the Steward method demonstrated clear differences in SIDapparent and Atot between control and the two haemorrhage and resuscitation treatments. However, in agreement with dog acid-base research, the clinical significance of this observation is not apparent (Torrente et al. 2014).

The free water effect indicated a dilutional acidosis for all time periods, except the next day, despite the pH not always being acidaemic (Hopper et al. 2014a). This finding could be attributed to the overall low sodium concentration reported in our cats, as previously described for SIDapparent. The free water effect was more negative during anaesthesia compared to the awake states, which suggests that this component of the semi-quantitative method would be a useful tool to help interpret the pH if our cats' sodium concentrations were within expected ranges (Hopper et al 2014a).

The chloride effect was different between control and the haemorrhage and resuscitation treatments. It could have resulted from the difference in haemoglobin concentration. The lower the circulating haemoglobin (anaemia), the lower the oxygen content in blood delivered to the metabolizing tissues which could have resulted in cellular hypoxia. Anaemia and cellular hypoxia stimulate an increased erythrocyte production of 2,3 diphosphoglyceric acid (2,3 DPG), an anionic allosteric effector that induced a conformational change in haemoglobin to facilitate the offloading oxygen to the tissues (Bunn 1980). However, cats do not make use of 2,3 DPG to increase the oxygen offloading capacity of haemoglobin (decreased oxygen affinity); instead they make use of chloride (Bunn 1980). The increase in erythrocyte chloride concentration causes the P50 (partial pressure of oxygen where 50% of the haemoglobin is saturated) to increase which effectively shifts the oxygen haemoglobin dissociation curve of the cat to the right (Bunn 1980). Furthermore, the decrease in haemoglobin concentration in LRS and Voluven could have altered the buffering capacity of haemoglobin within the blood stream and

could explain the difference in chloride effect because of the chloride shift where chloride moves into the erythrocyte and bicarbonate moves into the plasma (Hamburger phenomenon) (Higgins 2008). Considering these two chloride-mediated erythrocyte functions, it was expected that the chloride would move into the erythrocyte whereby decreasing the (or maintaining normal) concentration of chloride. However, counterintuitively, the haemoglobin concentration decreased profoundly and the corrected chloride increase over time during the resuscitation phase which suggests that the chloride concentration was in excess relative to the erythrocyte count (haemoglobin concentration).

The albumin effect was similar to the Atot of the Stewart method, which has already been discussed. The semi-quantitative method was the most informative of the three methods used to interpret the pH status of the cats. However, in agreement with Hopper et al. (2014a, b), the overall clinical relevance of these observations is not fully elucidated. Furthermore, the acidosis was not considered life threatening, even after severe haemorrhage and large-volume fluid resuscitation.

The study had notable limitations. The sample size was small and thus had the power to reliably detect only large effects; and even then, the magnitude of the effect is likely to be understated. This study is the first of its kind in cats and the observations herein will assist future researchers in estimating adequate samples sizes to validate these observations under various clinical conditions. The study was conducted in anaesthetised cats under controlled conditions. Therefore, extrapolation of our findings to trauma or awake patients or those receiving different drugs during anaesthesia will require further investigation.

4.6 Conclusions

Haemorrhage followed by large volume resuscitation did not alter the blood pH compared to a control treatment. However, despite the pH being similar over time among all treatments, there were notable differences in the variables used to interpret pH in the three methods between the control and the two treatments where haemorrhage was followed by fluid resuscitation. The major variables that changed were PvCO2, HCO_3^-(act) (Henderson-Hasselbalch); SIDapparent, Atot (Stewart); free water effect, chloride effect and albumin effect (semi-quantitative). The semi-quantitative method was subjectively more informative compared to the Henderson-Hasselbalch and Stewart methods but none was considered superior. Therefore, in clinical situations, perhaps familiarity with the three methods will allow for more informed decision-making during case management.

CHAPTER 5

Effects on coagulation during fluid resuscitation using lactated Ringer's solution or 6% tetrastarch 130/0.4 in controlled haemorrhaged anaesthetised cats

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Author contribution: Study design (Zeiler, Fuller, Dzikiti); Data collection (Zeiler, Buck, Pohlin); Data analysis (Zeiler, Kamerman); Manuscript drafting (Zeiler); Manuscript editing (All)

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5.1 Abstract

Objective To investigate coagulation processes in anaesthetised cats undergoing haemorrhage and resuscitation.

Study design Randomized controlled crossover study.

Animals Six healthy cats (21 ±1 months old; 4.9 ±1.2 kg).

Methods Blood samples for coagulation (prothrombin time [PT], activated partial thromboplastin time [aPTT], thromboelastography [TEG]) and haematology (haematocrit and platelet count) profiling were drawn from the jugular vein during a health check. The cats were anaesthetised (buprenorphine, alfaxalone, isoflurane in oxygen, fixed end-tidal concentration of 1.7%) and underwent three treatments as follows: 1) control, with mild haemorrhage and no resuscitation; 2) controlled haemorrhage and lactated Ringer's solution (LRS) for resuscitation; and 3) controlled haemorrhage and tetrastarch 130/0.4 (Voluven) for resuscitation. The LRS or Voluven were administered at 60- or 20-ml kg^{-1} $hour^{-1}$, respectively, for 120 minutes. Venous blood samples were collected for profiling immediately after haemorrhage (0) and at 60- and 120-minutes of fluid resuscitation; and a day after the treatment. Data were analysed using a general linear mixed model.

Results Total median blood loss at 120-minutes were 11.4, 31.0 and 30.8 ml kg^{-1} for control, LRS and Voluven, respectively. Prothrombin time and aPTT during LRS and Voluven were prolonged at time points 60- and 120-minutes compared to control ($p < 0.001$). On TEG; the reaction time, kinetic time and alpha-angle were within reference intervals for cats at all time points in all treatments; while maximum amplitude was less than the reference interval (40 mm) at time points 0-, 60- and 120-minutes during Voluven and at 60- and 120-minutes for LRS ($p < 0.001$). Haematocrit and platelet count were significantly lower at 60- and 120-minutes during LRS and Voluven compared to control ($p < 0.001$).

Conclusion and clinical relevance Coagulopathies were detected during haemorrhage and fluid resuscitation in anaesthetised cats. Prolongation of PT and aPPT and decreased clot strength may have been caused by haemodilution and loss of platelets.

5.2 Introduction

Cats are often anaesthetised for surgical procedures and the risk of intraoperative haemorrhage is always present. Intraoperative haemorrhage, if severe enough, will cause an absolute hypovolaemia and hypotension that may warrant resuscitation using fluids. Severe haemorrhage, in veterinary species, is defined as a blood loss of 40% or more and if the blood is lost acutely then compensatory mechanisms may fail and signs of haemorrhagic shock develop (Jutkowitz 2004). In cats, the blood volume ranges from 52.6 ± 6.8 to 59.6 ± 5.8 mL kg^{-1} (Groom et al. 1965; Mott 1968). Therefore, severe haemorrhage, in this study, was defined as a volume of blood loss exceeding 22 mL kg^{-1} of blood was lost. Mild haemorrhage is considered to be a volume of blood loss that does not require replacement and < 10 mL kg^{-1} (Jutkowitz 2004). Fluid therapy during anaesthesia in cats has been associated with increased odds of death (Brodbelt et al. 2007). Resuscitation fluids can be of either the isotonic crystalloid (crystalloids) or iso-oncotic colloid (colloids) kind (Driessen & Brainard 2006; Davis et al 2013). Conventional protocols of resuscitating the intravascular compartment to prevent haemorrhagic shock after an acute controlled haemorrhage are based on administration of shock rates or at ratios to the volume of blood being lost (Rozanski & Rondeau 2002). The shock rate dose protocol for administering crystalloids in cats range from 40 to 60 ml kg^{-1} $hour^{-1}$, whereas the dose rate for colloids are not established, especially for tetrastarches, but ranges from 5 to 15 ml kg^{-1} $hour^{-1}$ have been suggested (Rozanski & Rondeau 2002). The ratio dose protocol is where crystalloids are administered at a certain volume ratio to haemorrhaged blood and is commonly recommended to be at a ratio of 3:1 (the 3:1 rule), whereby 3 ml of crystalloid should be administered for every 1 ml of blood being lost. However, in human medicine, often larger volumes are required in the order of 4:1 to 5:1 to resuscitate the intravascular volume adequately (Rehm et al. 2019). The administration ratio for colloids is 1:1 (Broadstone 1999). The conventional resuscitation protocols are classified as being liberal fluid therapy.

Severe anaemia and administration of fluids at conventional resuscitation doses have been reported to cause coagulopathies in dogs *in vitro* (Brooks et al. 2014; Wurlod et al. 2015) and *in vivo* (Driessen & Brainard 2006), especially when colloids like hydroxyethyl starch fluids are administered (Adamik et al. 2015). However, the effect of haemorrhage or fluid administration on coagulation in cats is scantily reported in the literature (Adamik et al. 2015); instead, theories are extrapolated from dogs, for which the effects are presumed to be similar. Alwood et al. (2004) evaluated coagulation profiles of normal

healthy cats and found them to be “hypercoagulable” compared to dogs. Therefore, it may not be suitable to extrapolate information from dogs to cats. More recently, an *in vitro* study in which Ringer’s acetate and tetrastarch 6% 130/0.42 were mixed with feline fresh whole blood at a ratio of 1:6 and effects on global coagulation measured using rotational thromboelastometry found evidence of hypocoagulability. The tetrastarch 6% 130/0.42 caused a more significant hypocoagulable effect compared to the Ringer’s acetate fluid. However, the magnitude of the effects for both fluids were mostly within the reference interval of the laboratory and therefore the authors cautioned that the observations may be of limited clinical relevance (Albrecht et al. 2016).

Haemostasis is composed of a primary phase that overlaps with a secondary phase (Hyatt & Brainard 2016). Primary haemostasis is the phase of forming a platelet plug, typically after vascular damage. The secondary phase of haemostasis is when soluble clotting factors are activated to form a fibrin mesh that then infiltrates the platelet plug to form a firm, stable clot. Once the clot has served its function of repairing the blood vessel, it then dissolves through a process called fibrinolysis (Hyatt & Brainard 2016).

Conventional testing methods of coagulation include determining the prothrombin time (PT) and activated partial thromboplastin time (aPTT) (Hyatt & Brainard 2016). These methods test the

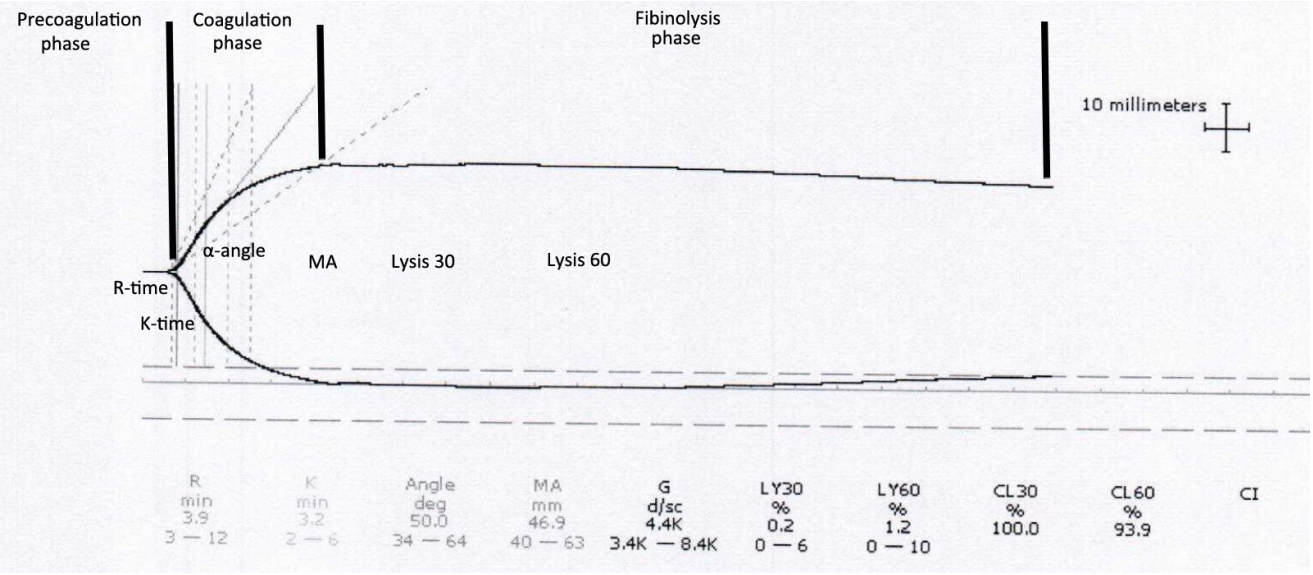


Figure 5.1 The thromboelastogram tracing consists of three phases which represent the different phases of coagulation, namely: the precoagulation phase, the coagulation phase and the fibrinolysis phase. Where: R-time: reaction time; K-time: kinetic time; α-angle: alpha-angle; MA: maximum amplitude; Lysis 30 (Ly30) and Lysis 60 (Ly 60) are the distances between the divergent lines at 30- and 60-minutes after reaching MA; G: global-value; CL30: clot lysis at 30 minutes; CL60: clot lysis at 60 minutes.

functioning of secondary haemostasis only, and therefore viscoelastic coagulation testing methods have been introduced, which assess global coagulation function (Brooks & Catalfamo 2013). Thromboelastography (TEG) is a viscoelastic coagulation testing method in which a tracing is plotted during coagulation and various parameters determined during the precoagulation, coagulation and fibrinolysis phases of haemostasis (**Figure 5.1**).

The conventional tests of coagulation have been used routinely for decades and therefore the pre-analytical and analytical methods are established and adhered to in practice (Hyatt & Brainard 2016). However, TEG is sensitive to many pre-analytical and analytical methodological differences and therefore incorrect clinical conclusions could be drawn from the outcomes of this test. Therefore, in an attempt to standardise testing, a team of experts (PROVETS: partnership on rotational viscoelastic test standardization) reviewed the literature and made recommendations for the entire testing process (Brainard et al. 2014; de Laforcade et al. 2014; Flatland et al. 2014; Goggs et al. 2014; Hanel et al. 2014; McMichael et al. 2014). These guidelines are essential to studies focusing on coagulation if meaningful conclusions are to be drawn and effective treatment recommendations are to be made to improve the outcome of treating coagulopathies in veterinary practice.

Guided by the PROVETS guidelines, the aims of the present study were to investigate the effects of haemorrhage followed immediately by resuscitation with an isotonic crystalloid (lactated Ringer's solution) or hydroxyethyl tetrastarch 6% 130/0.4 (Voluven) on coagulation in anaesthetised domestic healthy cats.

5.3 Materials and Methods

5.3.1 Animals

Six neutered domestic healthy cats (three males and three females; 21 ± 1-month old; 4.9 ± 1.2 kg) were used, based on availability, for the study. They were part of a colony housed in a communal indoor-outdoor cattery. The animal ethics committees of the University of Pretoria (v006-15) and University of

Witwatersrand (2017-10-68-C-AREC) approved the study. This study was part of a larger fluid resuscitation project and only data relevant to the present study are reported.

The cats underwent a health check assessment one week prior to the treatments through a clinical examination and coagulation and haematology profiling. No cats were excluded from participating in the study following the pre-treatment evaluation. Food, but not water, was withheld eight hours prior to treatment.

5.3.2 Study procedures

Cats were randomly allocated (balanced single block design; www.randomization.com) to receive three treatments at 2 months intervals. On the morning of treatment, the cat was transferred to the theatre complex of the hospital where it underwent a brief clinical examination and was weighed. The cat was premedicated with buprenorphine hydrochloride (0.02 mg kg^{-1}; Temgesic 0.3 mg ml^{-1}; Reckitt Benckiser Healthcare) intramuscularly and left undisturbed for 45 minutes. The cat was then transferred to the induction room and an indwelling catheter (22 gauge; Jelco; Smith Medical International) was aseptically inserted into one of the cephalic veins and secured. Alfaxalone (Alfaxan-CD RTU 10 mg ml^{-1}; Afrivet) was administered intravenously to induce general anaesthesia. The trachea was intubated with a cuffed endotracheal tube, which was secured in place and connected to a paediatric circle breathing system (15 mm internal diameter). General anaesthesia was maintained by delivering isoflurane (Isofor; Safeline Pharmaceuticals) in oxygen at a fixed flow rate of 80 ml kg^{-1} $minute^{-1}$ with the vaporiser initially set at 2%. A target end-tidal isoflurane concentration was standardised for the study and maintained at 1.7% throughout the procedures (Shaughnessy & Hofmeister 2014). The ventral neck region was clipped and surgically scrubbed before the cat was transferred to the surgery theatre.

Once in theatre the cat was placed in dorsal recumbency and connected to the anaesthetic machine, as previously described. A crystalloid (lactated Ringer's solution; Fresenius Kabi) was infused by an electronic device (Infusomat Space; B-Braun; South Africa) at 5 ml kg^{-1} $hour^{-1}$ via a y-ported administration set connected to the cephalic catheter for peri-anaesthetic maintenance fluids

(maintenance fluids). Probes and leads were placed and connected to a multiparameter machine (Datex-Ohmeda Cardiocap 5; GE healthcare; Finland) to monitor physiological variables, as follows: heart rate by electrocardiogram, peripheral oxygen haemoglobin saturation by pulse oximetry, end-tidal carbon dioxide ($PE'CO_2$) and respiratory rate by capnography, and oesophageal temperature. The end-tidal isoflurane concentration was monitored and the vaporiser adjusted to maintain a standardised concentration. A catheter (22 Gauge, 50 mm, Arrow arterial catheterization set; Arrow International) was inserted into the right pre-superficialised (superficialised 15 months earlier during gonadectomy) carotid artery by cut-down technique to allow for invasive blood pressure monitoring and intermittent blood sampling. A catheter (22 Gauge, 50 mm, Arrow arterial catheterization set) was inserted percutaneously into the left jugular vein for intermittent blood sampling and facilitation of controlled haemorrhage later. Both catheters were inserted by the Seldinger technique (Seldinger 1953). Then, one of the three randomly allocated treatments was commenced. The treatments were divided into two phases, the haemorrhage phase and resuscitation phase, as follows:

Treatment Control: The cat underwent a waiting period of 15 minutes to simulate controlled haemorrhage followed by a sham resuscitation phase of 120 minutes duration.

Treatment lactated Ringer's solution (LRS): The cat underwent a controlled haemorrhage phase until an end-point was reached (see below), followed by a resuscitation phase whereby a crystalloid (LRS) was infused at 60 ml kg^{-1} $hour^{-1}$ for 120 minutes.

Treatment Voluven: The cat underwent a controlled haemorrhage phase until an end-point was reached (see below), followed by a resuscitation phase whereby a colloid (Voluven; 6% tetrastarch 130/0.4; Fresenius Kabi) was infused at 20 ml kg^{-1} $hour^{-1}$ for 120 minutes.

During the haemorrhage phase blood was withdrawn manually into a semi-closed system using 20 ml syringes primed with citrate-phosphate-dextrose (4 mL; JMS blood bag, 450 mL; JMS Singapore) via the jugular catheter at a targeted rate of 2 ml kg^{-1} $minute^{-1}$ (targeted 15 minute haemorrhage phase) until one of two endpoints. The endpoint was either a 1) maximum blood withdrawal of 30 ml kg^{-1} or 2) mean arterial blood pressure of < 48 mmHg that persisted for at least 3 minutes, whichever happened first.

During the resuscitation phase the randomised resuscitation fluid was infused via a second electronic device (Infusomat Space), where its administration set was connected to the y-port of the

maintenance fluid administration set. Both fluids were administered simultaneously in LRS and Voluven, and control only received maintenance fluids.

Blood samples were obtained and physiological variables recorded at fixed time-points during the haemorrhage and resuscitation phases. On completion of the resuscitation phase; the monitoring equipment and catheters, except the jugular catheter, were removed. The carotid artery catheter was removed and digital pressure was applied until a stable clot was formed. If a stable clot did not form within 15 minutes then a haemostatic absorbable mesh (BloodStop iX; Life Science Plus; USA, CA) was applied using digital pressure for 10 minutes, or until haemostasis was achieved. Then the cut-down incision site was sutured using a two-layer closure technique with absorbable suture material (MonoPlus 5/0; B Braun). The cat received a single subcutaneous injection of meloxicam (0.2 mg kg^{-1}; 5 mg mL^{-1} Petcam; CiplaVet) and intravenous injection of buprenorphine (0.03 mg kg^{-1}). The cat was then transferred to the intensive care unit of the hospital for recovery and overnight observation without any further treatments or interventions required. The next day (24 hours later), blood was sampled and the jugular catheter was removed before the cat was transferred back to the cattery. All cats were rehomed, without evidence of renal or other organ injury or dysfunction, through an adoption process one month after conclusion of the study.

5.3.3 Data collection and analysis

Data relevant to the present study were collected in the form of coagulation and haematology profiling during the health check, during the treatment period (immediately after haemorrhage phase [0], at 60- and 120- minutes) and the next day. However, additional blood samples were collected at 30- and 90-minutes and are not reported here but included in the total blood loss calculation. The total blood loss at each time point was cumulative and included the controlled haemorrhage volume (not applicable in control) and all blood samples for profiling. The total volume of fluids that were administered during resuscitation were used for reporting because all cats were administered maintenance fluids during all treatments and that volume (10 ml kg^{-1} over 120 minutes) was considered negligible.

Venous blood samples were drawn from the jugular vein by direct puncture using a needle and syringe and decanted into two different storage tubes (sodium citrate 0.109M and Ethylenediaminetetraacetic acid tubes; BD Vacutainers; BD, Becton Dickinson and Company; Plymouth, UK) during the health check; or via the jugular catheter into a syringe and immediately decanted into the storage tubes during the treatment period and next day. Before the collection of a blood sample from the catheter, a small portion of waste blood (1.5 ml) was discarded. The sodium citrate tube was filled first in order of collection and to 2.7 ml, as recommended by the manufacturer. The stored blood was submitted to the onsite laboratory immediately for handling and measurement of PT, aPTT (ACL Elite, Werfen, Barcelona, Spain) and TEG (Haemoscope TEG 5000 Thrombelastograph Hemostasis Analyzer, Haemonetics, Braintree, Massachusetts, United States) and haematology (haemoglobin concentration, haematocrit, red cell count and platelet count; Advia 2120, Siemens Healthineers, Erlangen, Germany) using daily-calibrated machines, operated by experienced veterinary clinical technologists. Control and treatment PT and aPTT assays were run concurrently under the same conditions for standardisation purposes. The sodium citrate sample was rested for 30 minutes at room temperature before TEG analysis, using feline tissue factor as the activator, and the test was run at 37 °C for 120 minutes.

The data were tested for normality by plotting histograms, evaluating descriptive statistics and performing the Anderson-Darling test for normality. Data were nonparametric and therefore reported as median (interquartile range). All variables were compared among treatments and over time using a general linear mixed model and significant findings underwent post-hoc Tukey pairwise comparisons using Bonferroni correction for repeated measures. Model fits were assessed by visually inspecting residual plots to assess linearity, homogeneity of variances, normality, and outliers. Data were analysed using a commercially available software (MiniTab 18; Minitab Inc; USA, PA) and statistical significance set at $p < 0.05$.

5.4 Results

The amount of blood lost at 60-minutes was 7.6 (4.9 to 7.8), 27.3 (17.1 to 33.5) and 27.0 (19.2 to 30.4) ml kg^{-1} for control, LRS and Voluven, respectively. The total volume of fluids administered for

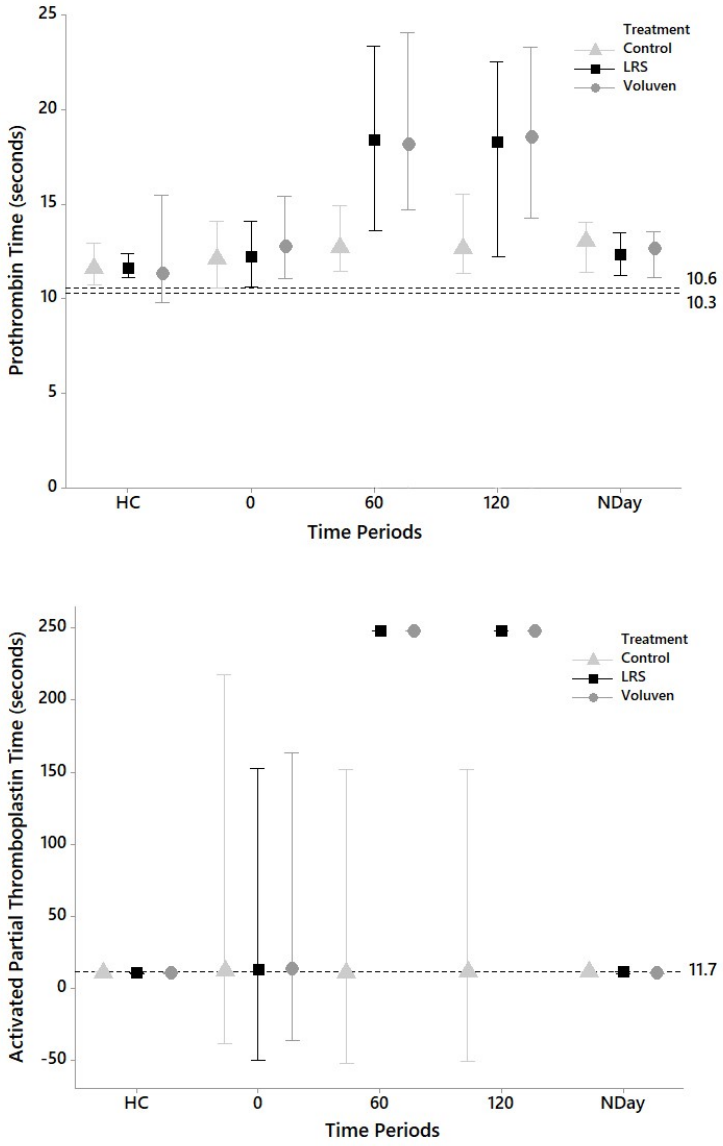


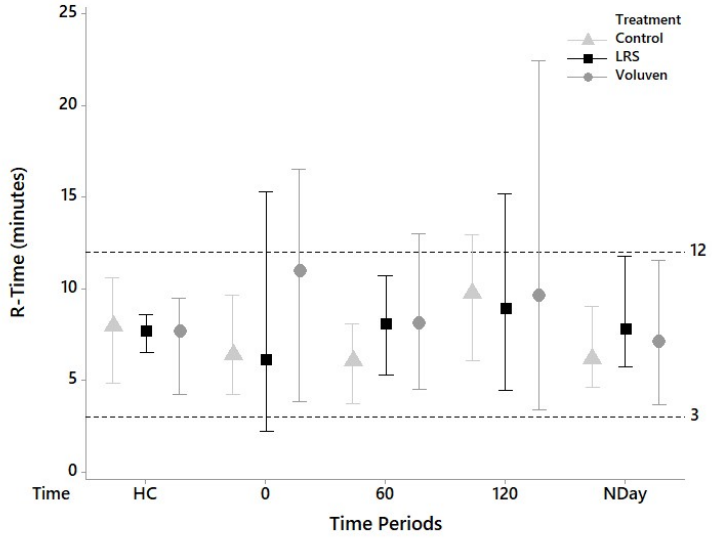
Figure 5.2 Confidence interval plots (95%) of prothrombin time and activated partial prothrombin time measured at the health check (HC), immediately after the haemorrhage phase (0), at 60- and 120-minutes during the resuscitation phase and the day after the study procedures (NDay). The isoflurane in oxygen anaesthetised cats underwent three treatments, as follows: 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. The dashed lines represent the upper and lower reference interval for the test published by the clinical pathology laboratory where the tests were done.

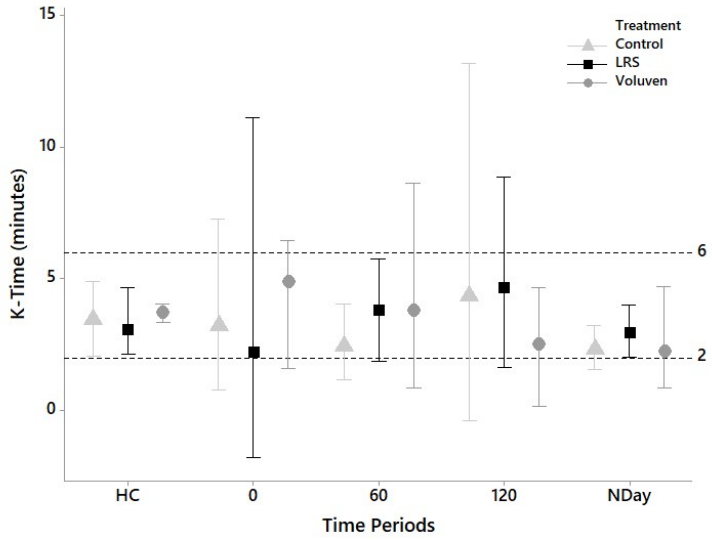
resuscitation at 60-minutes was 0-, 60- and 20-ml kg^{-1} for control, LRS and Voluven, respectively. The ratio of blood to fluid administered were approximately 2.2:1 and 0.7:1 for LRS and Voluven, respectively. The total blood lost at 120-minutes was 11.4 (7.4 to 11.7), 31 (19.4 to 37.6) and 30.8 (21.6 to 34.8) ml kg^{-1} for control, LRS and Voluven, respectively. The total volume of fluids administered for resuscitation at 120-minutes was 0-, 120- and 40-ml kg^{-1} for control, LRS and Voluven, respectively. The ratio of blood to fluid administered were approximately 3.9:1 and 1.3:1 for LRS and Voluven, respectively. The PT and aPTT results are summarised in **Table 5.1** and **Figure 5.2**. The PT was significantly longer during LRS and Voluven at time period 60- and 120-minutes compared to all other time points but not different amongst the treatments (time: p = 0.028; F = 2.88). The aPTT was prolonged during LRS and Voluven at time points 60- and 120-minutes compared to all other time points and different to control (treatment: p < 0.001; F = 14.59 || time: p < 0.001; F = 37.98 || Interaction: treatment x time: p < 0.001; F = 7.45).

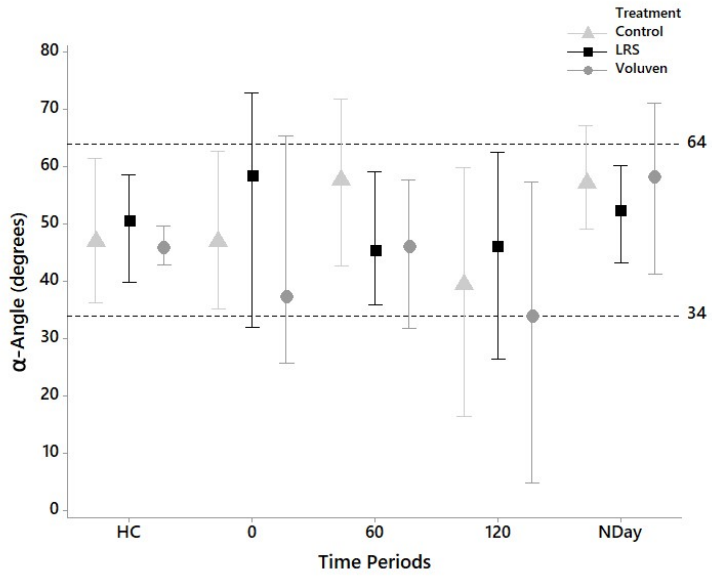
Table 5.1 Prothrombin time (PT) and activated partial thromboplastin time (aPTT) of isoflurane in oxygen anaesthetised cats undergoing three treatments. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation.

Parameter	**Unit**	**Treatment**	**Time period**									
			HC		**0**		**60**		**120**		**NDay**	
			$\tilde{x}$	**(IQR)**	$\tilde{x}$	**(IQR)**	$\tilde{x}$	**(IQR)**	$\tilde{x}$	**(IQR)**	$\tilde{x}$	**(IQR)**
PT	Seconds	Control	11.6	(10.9; 12.8)	12.1	(11.4; 13.1)	12.7	(12.1; 14.3)	12.7	(12.2; 14.6)	13.0	(11.7; 13.6)
		LRS	11.6	(11.3; 12.4)	12.2	(11.0; 13.8)	**18.4***	(17.2; 58.5)	**18.3***	(15.2; 58.0)	12.3	(11.4; 13.2)
		Voluven	11.3	(10.9; 14.8)	12.8	(12.1; 14.1)	**18.1***	(15.6; 57.5)	**18.5***	(15.7; 170.0)	12.7	(11.2; 13.4)
aPTT	Seconds	Control	10.6	(10.3; 11.5)	11.8	(10.3; 246.1)	**10.6†**	(10.3; 70.0)	**11.3†**	(10.5; 72.9)	11.2	(10.6; 11.8)
		LRS	10.9	(10.3; 11.3)	12.7	(10.4; 72.2)	**247.5*†**	(247.5; 247.5)	**247.5*†**	(247.5; 247.5)	11.4	(10.3; 11.7)
		Voluven	10.4	(10.3; 11.1)	13.3	(11.6; 126.5)	**247.5*†**	(247.5; 247.5)	**247.5*†**	(247.5; 247.5)	10.5	(10.3; 10.7)
			Control measurements									
Con PT	Seconds	Control	10.5	(10.3; 10.6)	10.5	(10.1; 10.6)	10.5	(10.1; 10.6)	10.5	(10.1; 10.6)	10.5	(10.1; 10.6)
		LRS	10.5	(10.3; 10.6)	10.5	(10.1; 10.6)	10.5	(10.1; 10.6)	10.5	(10.1; 10.6)	10.5	(10.1; 10.6)
		Voluven	10.5	(10.3; 10.6)	10.5	(10.1; 10.6)	10.5	(10.1; 10.6)	10.5	(10.1; 10.6)	10.5	(10.1; 10.6)
Con aPTT	Seconds	Control	11.5	(11.1; 11.8)	11.7	(11.3; 11.9)	11.7	(11.3; 11.9)	11.7	(11.3; 11.9)	11.7	(11.3; 11.9)
		LRS	11.5	(11.1; 11.8)	11.7	(11.3; 11.9)	11.7	(11.3; 11.9)	11.7	(11.3; 11.9)	11.7	(11.3; 11.9)
		Voluven	11.5	(11.1; 11.8)	11.7	(11.3; 11.9)	11.7	(11.3; 11.9)	11.7	(11.3; 11.9)	11.7	(11.3; 11.9)

$\tilde{x}$: median; (IQR): interquartile range; Bold numbers: values outside of the reference interval; *: significant value ($p < 0.05$) over time; †: significant value ($p < 0.05$) among treatments; HC: health check; 0: time period immediately after haemorrhage; 60 and 120: 60 and 120 minutes after haemorrhage and during fluid resuscitation; NDay: next day; CON: control measurements.







The TEG values for the measured variables are summarised in **Table 5.2**. The R-time did not differ among treatments or over time. Similarly, K-time did not differ among treatments or over time. The α-Angle was significantly different over time only in cats receiving the control and Voluven treatments, but not LRS (time: p = 0.013; F = 3.40). Despite the significant differences in α-Angle, their median values were all within the laboratory reference intervals for cats (**Figure 5.3**).

Figure 5.3 Confidence interval plots (95%) of the R-time, K-time and alpha angle measured by thromboelastography over time. Samples were collected at the health check (HC), immediately after the haemorrhage phase (0), at 60- and 120-minutes during the resuscitation phase and the day after the study procedures (NDay). The isoflurane in oxygen anaesthetised cats underwent three treatments, as follows: 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. The dashed lines represent the upper and lower reference interval for the test published by the clinical pathology laboratory where the tests were done.

Table 5.2 Thromboelastography variables measured in isoflurane in oxygen anaesthetised cats undergoing three treatments. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} hour^{-1}, respectively, for resuscitation.

Parameter	Unit	Treatment	Time period									
			HC		0		60		120		NDay	
			$\tilde{x}$	(IQR)	$\tilde{x}$	(IQR)	$\tilde{x}$	(IQR)	$\tilde{x}$	(IQR)	$\tilde{x}$	(IQR)
R-time	minutes	Control	7.95	(5.15; 10.10)	6.40	(4.92; 9.88)	6.05	(4.03; 7.98)	9.75	(7.00; 11.57)	6.20	(5.25; 8.75)
		LRS	7.70	(6.90; 8.40)	6.15	(4.98; 12.77)	8.10	(6.20; 9.85)	8.90	(5.50; 13.20)	7.80	(6.92; 11.70)
		Voluven	7.70	(3.88; 8.70)	11.00	(5.35; 14.70)	8.15	(5.63; 11.05)	9.65	(6.92; 19.23)	7.15	(4.65; 9.52)
K-time	minutes	Control	3.45	(2.28; 4.93)	3.20	(1.87; 5.53)	2.45	(1.40; 4.00)	4.35	(3.45; 8.38)	2.30	(1.73; 2.90)
		LRS	3.05	(2.63; 4.25)	2.20	(1.78; 6.47)	3.80	(2.30; 5.35)	4.65	(2.08; 8.48)	2.95	(2.18; 3.88)
		Voluven	3.70	(3.43; 3.98)	4.90	(2.00; 5.70)	3.80	(2.30; 6.57)	2.50	(0.00; 4.53)	2.25	(1.45; 3.90)
α-Angle	deg	Control	46.80	(37.95; 59.55)	46.95	(39.05; 63.47)	**57.60***	(44.17; 69.40)	**39.40***	(28.93; 48.15)	**57.05***	(53.10; 66.70)
		LRS	50.40	(42.45; 55.40)	58.25	(42.42; 65.85)	45.30	(39.00; 57.05)	45.90	(28.40; 61.60)	52.25	(43.88; 59.00)
		Voluven	45.75	(43.25; 49.70)	**37.20***	(32.40; 62.80)	45.90	(35.02; 55.60)	**33.90***	(3.20; 56.10)	**58.20***	(46.82; 68.30)
MA	mm	Control	47.60	(44.30; 56.15)	45.60	(33.15; 52.10)	50.95	(40.08; 59.77)	42.30	(34.13; 49.35)	51.95	(48.03; 57.60)
		LRS	46.60	(40.30; 56.35)	48.85	(41.35; 53.35)	**37.20***	(28.05; 45.55)	**34.80***	(27.05; 47.57)	51.45	(46.25; 54.70)
		Voluven	44.40	(40.83; 52.88)	**39.60***	(35.80; 47.35)	**36.80***	(30.32; 44.02)	**37.00***	(2.58; 41.73)	55.55	(47.70; 59.02)
G-value	kdyn sec^{-1}	Control	4.55	(3.98; 6.58)	4.20	(2.50; 5.48)	5.45	(3.35; 7.43)	3.65	(2.63; 4.95)	5.40	(4.70; 6.83)
		LRS	4.35	(3.43; 6.53)	4.75	(3.85; 5.73)	**3.00***	(1.95; 4.20)	**2.65***	(1.85; 4.55)	5.30	(4.28; 6.03)
		Voluven	4.00	(3.43; 5.60)	**3.30***	(2.75; 4.55)	**2.90***	(2.20; 3.98)	**2.95***	(0.10; 3.60)	6.25	(4.63; 7.30)
Lysis 30	%	Control	0.80	(0.15; 3.3.8)	3.25	(0.40; 4.23)	5.20	(1.28; 7.17)	1.50	(0.90; 3.70)	2.85	(0.18; 3.80)
		LRS	0.00	(0.00; 2.33)	2.95	(0.00; 7.67)	6.80	(3.15; 10.10)	6.10	(1.42; 11.50)	1.40	(0.00; 13.78)
		Voluven	1.05	(0.15; 2.83)	3.20	(1.10; 27.05)	4.10	(2.75; 17.52)	4.65	(0.00; 14.80)	2.30	(0.33; 3.18)
Lysis 60	%	Control	4.75	(2.65; 7.95)	9.00	(4.47; 11.37)	12.05	(5.20; 14.90)	5.90	(4.95; 9.70)	7.80	(3.65; 9.32)
		LRS	2.75	(0.38; 6.25)	4.05	(0.75; 10.98)	14.90	(8.15; 19.65)	12.25	(6.45; 19.50)	6.60	(0.60; 22.48)
		Voluven	5.30	(0.92; 6.73)	8.50	(5.70; 36.90)	11.40	(9.10; 28.23)	11.60	(0.00; 23.38)	6.85	(4.63; 8.92)

$\tilde{x}$: median; (IQR): interquartile range; Bold numbers: values outside of reference interval; *: significant value ($p < 0.05$) over time; †: significant value ($p < 0.05$) among treatments; HC: health check; 0: time after haemorrhage phase but before commencement of resuscitation phase; 60: 60 minutes into the resuscitation phase; 120: 120 minutes which was the end of the resuscitation phase; NDay: the following day; R-time: reaction time; K-time: kinetic time; deg: degrees; MA: maximum amplitude; G-value: global clot index; kdyn sec^{-1}: kilodynes per second; Lysis 30 and 60: clot lysis at 30 and 60 minutes after MA represented as a percentage of MA.

The MA was significantly smaller during LRS at time points 60- and 120-minutes and during Voluven at time points 0-, 60- and 120-minutes compared to all other time points, but not different amongst the treatments (time: $p < 0.001$; F = 6.89). The G-value varied similarly to the MA and also differed over time, but not amongst treatments (time: $p < 0.001$; F = 6.39). The significant values in LRS and Voluven over time for the MA and G-value were smaller than the lowest limit of the laboratory reference intervals for cats (**Figure 5.4**). There were no differences over time or among treatments for lysis 30 and lysis 60. However, median values and ranges were outside the limits of the laboratory reference intervals for cats after administration of LRS and Voluven over time, compared to control (**Figure 5.5**).

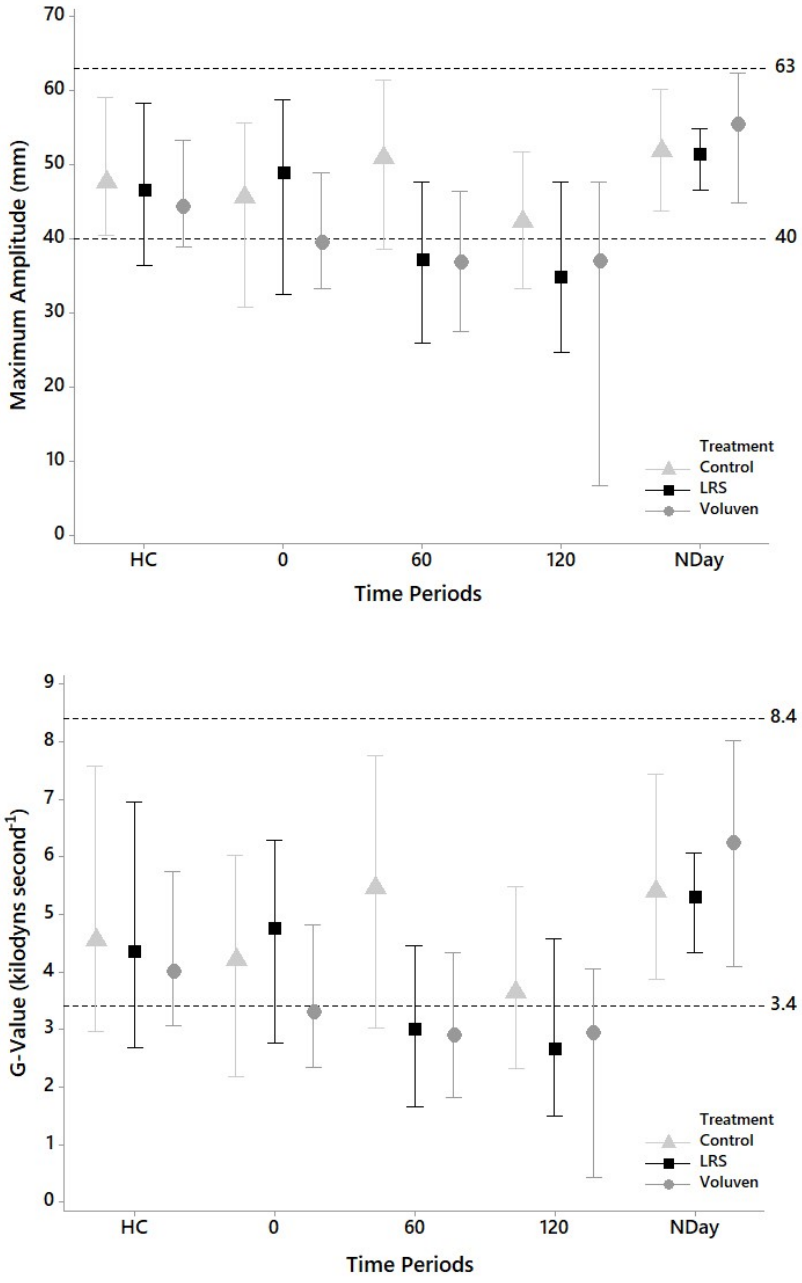


Figure 5.4 Confidence interval plots (95%) of the maximum amplitude and G-value measured by thromboelastography over time. Samples were collected at the health check (HC), immediately after the haemorrhage phase (0), at 60- and 120-minutes during the resuscitation phase and the day after the study procedures (NDay). The isoflurane in oxygen anaesthetised cats underwent three treatments, as follows: 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. The dashed lines represent the upper and lower reference interval for the test published by the clinical pathology laboratory where the tests were done.

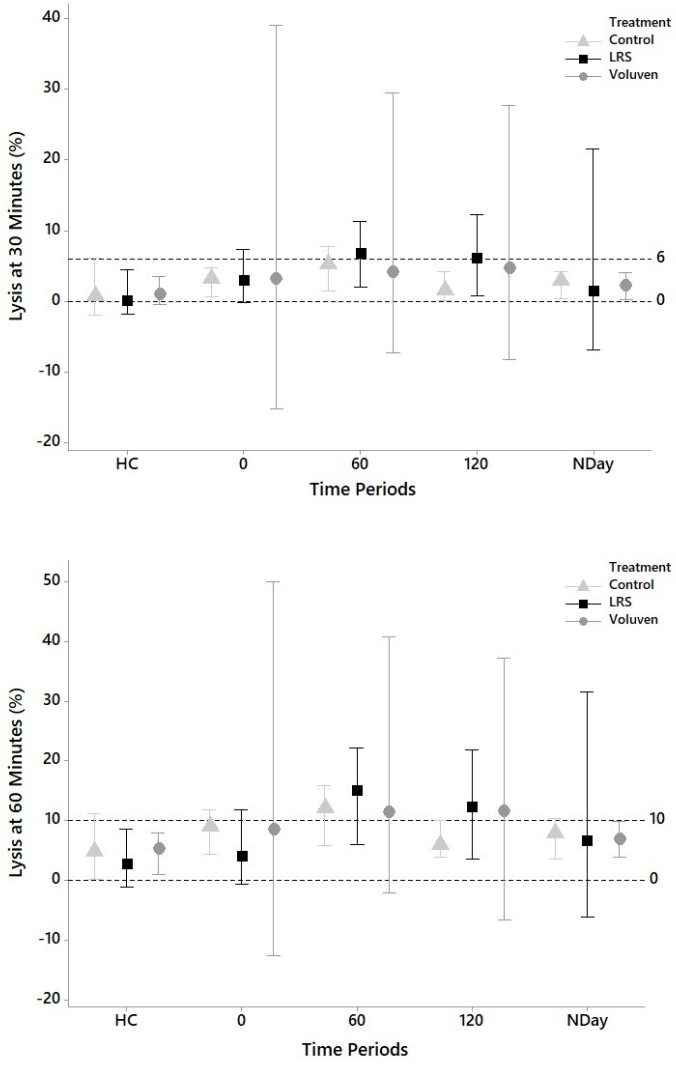


Figure 5.5 Confidence interval plots (95%) of clot lysis at 30 and 60 minutes measured by thromboelastography over time, respectively. Samples were collected at the health check (HC), immediately after the haemorrhage phase (0), at 60- and 120-minutes during the resuscitation phase and the day after the study procedures (NDay). The isoflurane in oxygen anaesthetised cats underwent three treatments, as follows: 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation. The dashed lines represent the upper and lower reference interval for the test published by the clinical pathology laboratory where the tests were done.

Results on haematological variables are summarised in **Table 5.3**. The red cell count was significantly lower during LRS and Voluven at time points 60- and 120-minutes compared to other time points and also significantly different to the control (treatment: $p < 0.001$; F = 14.38 || time: $p < 0.001$; F = 73.94 || Interaction: treatment x time: $p < 0.001$; F = 8.35). Haemoglobin concentration and haematocrit changed in a similar way to the red cell count ([treatment: $p < 0.001$; F = 35.20 || time: $p < 0.001$; F = 133.18 || Interaction: treatment x time: $p < 0.001$; F = 11.49] and [treatment: $p < 0.001$; F = 21.32 || time: $p < 0.001$; F = 113.25 || Interaction: treatment x time: $p < 0.001$; F = 12.40], respectively). The platelet count was significantly lower during LRS and Voluven at time points 60- and 120-minutes compared to other time points and different to control (treatment: $p = 0.001$; F = 7.94 || time: $p < 0.001$; F = 12.06). The significantly different values for the haematological variables were lower than the laboratory reference interval for cats (**Figure 5.6**).

Table 5.3 Haemoglobin concentration (Hb), red cell count (RCC), haematocrit (Ht) and platelet count in isoflurane in oxygen anaesthetised cats undergoing three treatments. The treatments were 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} $hour^{-1}$, respectively, for resuscitation.

Parameter	**Unit**	**Treatment**	**Time period**									
			HC		**0**		**60**		**120**		**NDay**	
			$\tilde{x}$	**(IQR)**	$\tilde{x}$	**(IQR)**	$\tilde{x}$	**(IQR)**	$\tilde{x}$	**(IQR)**	$\tilde{x}$	**(IQR)**
Hb	g L^{-1}	Control	127	(123;149)	**83***	(79; 87)	**90**†	(84; 105)	**88**†	(84; 92)	110	(94; 117)
		LRS	134	(126; 136)	**97***	(88; 107)	**46***†	(42; 51)	**43***†	(37; 58)	**79***	(76; 101)
		Voluven	135	(122; 142)	**91***	(84; 104)	**44***†	(37; 49)	**33***†	(29; 41)	**89***	(79; 93)
RCC	x$10^{12}L^{-1}$	Control	8.2	(7.9; 8.8)	**5.7***	(5.3; 6.6)	**6.2***†	(5.4; 7.2)	**6.0***†	(5.3; 6.6)	7.2	(6.5; 8.0)
		LRS	8.8	(8.2; 9.5)	**6.6***	(6.1; 7.8)	**3.4***†	(3.0; 3.6)	**3.0***†	(2.7; 4.2)	**6.3***	(5.4; 7.4)
		Voluven	8.8	(98.1; 10.0)	**6.4***	(5.4; 7.7)	**3.0***†	(2.7; 3.5)	**2.4***†	(2.1; 2.8)	**5.9***	(5.5; 6.2)
Ht	L L^{-1}	Control	0.38	(0.35; 0.40)	**0.27***	(0.25; 0.27)	**0.27***†	(0.25; 0.31)	**0.27***†	(0.26; 0.29)	0.34	(0.29; 0.35)
		LRS	0.40	(0.37; 0.42)	**0.30***	(0.28; 0.33)	**0.15***†	(0.14; 0.16)	**0.13***†	(0.12; 0.19)	**0.28***	(0.24; 0.35)
		Voluven	0.40	(0.37; 0.45)	**0.28***	(0.27; 0.32)	**0.14***†	(0.13; 0.15)	**0.11***†	(0.09; 0.13)	**0.29***	(0.25; 0.29)
Platelet	x$10^{9}L^{-1}$	Control	356	(250; 431)	344	(277; 395)	**290***†	(265; 348)	**281***†	(236; 352)	**281***	(228; 327)
count		LRS	359	(299; 434)	**272***	(164; 337)	**193***†	(162; 226)	**178***†	(152; 233)	**225***	(196; 304)
		Voluven	338	(237; 403)	335	(294; 377)	**199***†	(135; 247)	**150***†	(121; 181)	**271***	(191; 282)

$\tilde{x}$: median; (IQR): interquartile range; Bold numbers: values out of reference interval; *: significant value ($p < 0.05$) over time; †: significant value ($p < 0.05$) among treatments; HC: health check; 0: time after haemorrhage phase but before commencement of resuscitation phase; 60: 60 minutes into the resuscitation phase; 120: 120 minutes which was the end of the resuscitation phase; NDay: the following day.

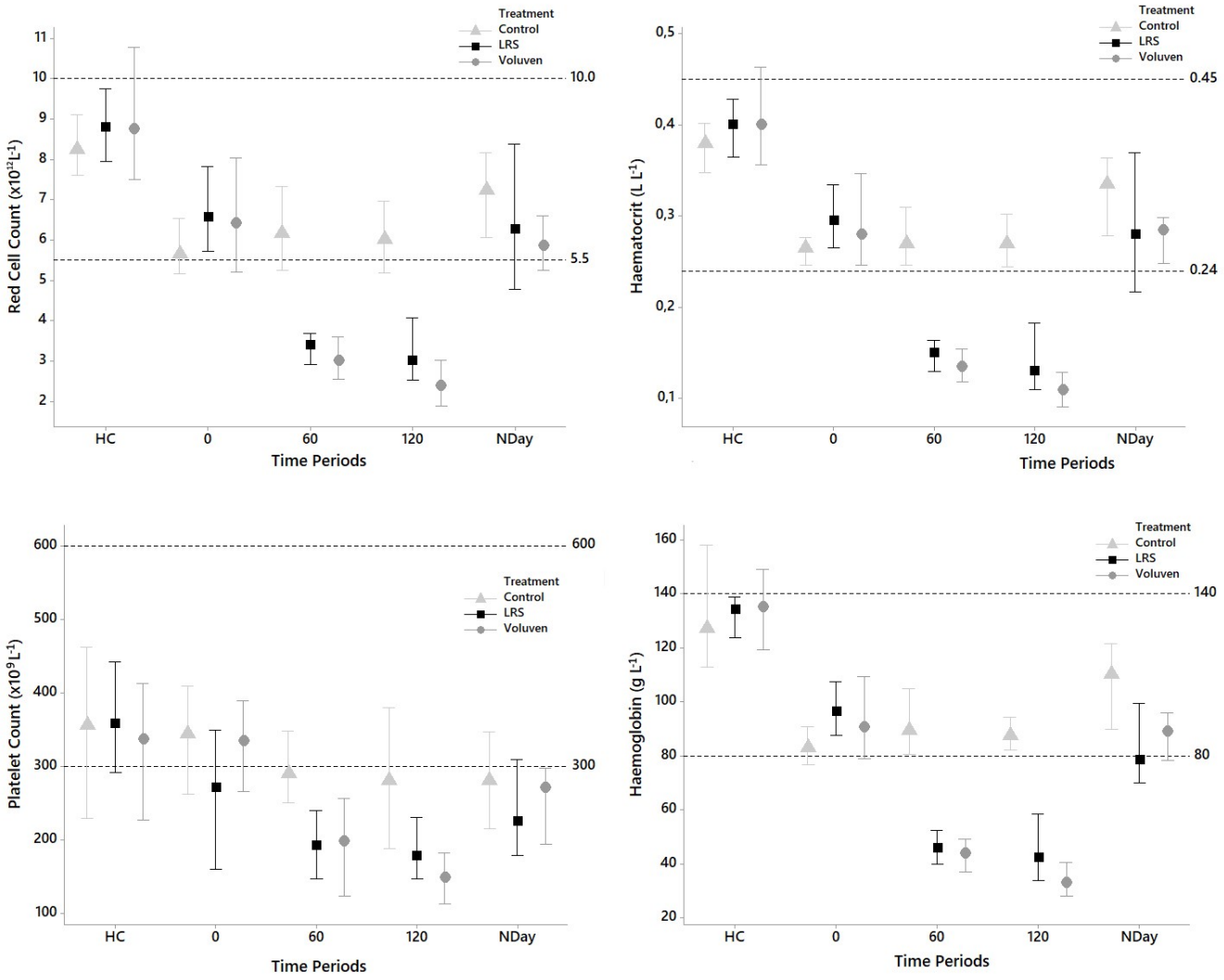


Figure 5.6 Confidence interval plots (95%) of red cell count, platelet count, haematocrit and haemoglobin concentration measured over time. Samples were collected at the health check (HC), immediately after the haemorrhage phase (0), at 60- and 120-minutes during the resuscitation phase and the day after the study procedures (NDay). The isoflurane in oxygen anaesthetised cats underwent three treatments, as follows: 1) control where mild haemorrhage (< 10 mL kg^{-1}) and no fluid resuscitation occurred, or controlled severe haemorrhage (> 22 mL kg^{-1}) followed by 2) lactated Ringer's solution (LRS), and 3) 6% tetrastarch 130/0.4 (Vol) infusion at 60- or 20 mL kg^{-1} hour^{-1}, respectively, for resuscitation. The dashed lines represent the upper and lower reference interval for the test published by the clinical pathology laboratory where the tests were done.

5.5 Discussion

Overall, coagulation profile was normal in the cats during their awake states (health check and the day after haemorrhage) and after the haemorrhage phase during all treatments. Coagulation derangements, however, were present when the cats received the two resuscitation fluids after haemorrhage, but not in the control where they had mild haemorrhage and no resuscitation fluid. The conventional methods of testing coagulation, PT and aPTT, detected derangements. The TEG detected derangements during the coagulation (specifically MA) and fibrinolysis phases (specifically lysis 60) of the tracing. The outcome of the study was counterintuitive in that the conventional testing methods detected abnormalities thought to be related to coagulation factors. In contrast, the TEG detected abnormalities thought to be related to haematocrit and platelet counts.

Hypocoagulability and hypercoagulability have not been defined in cats because there is insufficient evidence to allow the formulation of such definitions (Goggs et al. 2014). The conventional methods only test for derangements in secondary haemostasis and only detect profound derangements (Hyatt & Brainard 2016). These conventional tests cannot be used alone to define a hypo- or hypercoagulable state, but may provide information about hypocoagulability and can be useful as screening tests to identify a coagulopathy (Donahue & Otto 2005; Brooks & Catalfamo 2013). The TEG, which assesses global coagulation function, has trace variables that can indicate hypocoagulability, including: prolongation in R-times and K-times, and decreases in α-angle, MA and G-values compared to laboratory reference intervals (and vice versa for a hypercoagulable state; Goggs et al. 2014). The lack of a consensus definition of hypo- and hypercoagulability has encouraged the recommendation to publish as many test variables to allow for post-hoc assessment and definition of coagulability (Hanel et al. 2014). Furthermore, the quality of the published data must meet current standardised pre-analytical and analytical standards to allow for future meta-analysis studies (Hanel et al. 2014). Our clinical pathology laboratory adhered to current practice recommendations and its reference range intervals are similar to those of other cat studies (Alwood et al. 2004; Marschner et al. 2010; Cuq et al. 2017).

The prolonged PT and aPTT could have arisen because of haemodilution, whereby all coagulation factors were diluted out enough to cause the derangement (Driessen & Brainard 2006; Brooks &

Brainard 2013; Adamik et al. 2015). Indeed, the derangements were similar in cats during LRS or Voluven in the present study. The effects of LRS and Voluven were especially profound in the aPTT, a test that assesses the intrinsic and common pathways (Donahue & Otto 2005). The R-time of the TEG provides a similar measure, but unexpectedly its value remained within the cat reference interval in all treatment groups at all time points. We expected to observe a prolonged R-time at time points 60- and 120-minutes during LRS and Voluven. The unexpected difference in these two test results could possibly be attributed to the use of different reagents and activators in aPPT and TEG assays, because if dilution was the only factor then we would have expected similar findings. However, in anaemic dogs, the results of viscoelastic tests of coagulation have indicated that they have a hypercoagulability identified by short R-time, short K-time and increased MA (Smith et al. 2012; Brooks et al. 2014). That is similar to our cats, which had a shorter than expected R-time at time points 60- and 120-minutes during LRS and Voluven when the haematocrits were at their lowest. However, MA in our cats, at these time points, was lower than the lowest reference interval level, unlike reports on anaemic dogs. The low MA in our cats could be explained by the decrease in the platelet count at these time points. The platelet counts were corrected and standardised in the *in vitro* dog study (Brooks et al. 2014) or positively correlated with maximum clot firmness (a thromboelastometry variable similar to MA) in the *in vivo* dog study (Smith et al. 2012). The low haematocrit, however, is not the only factor related to a hypercoagulability, the overall blood viscosity also contributes to this observation. The viscosity of whole blood is mainly attributed to the red cell mass under normal physiological condition, but plasma proteins, especially fibrin, also contribute to viscosity (Brook et al. 2013). When there are a low haematocrit and low blood viscosity then the artificial hypercoagulability occurs; whereas a low haematocrit and normal viscosity cause a hypocoagulability (Brook et al. 2013).

Coagulopathies were detected at time point 60 minutes, which translates to total volumes of LRS and Voluven infused at this time point of 60- and 20-ml kg^{-1}, respectively. The approximate administration ratios were 2.2:1 and 0.7:1 for LRS and Voluven, respectively to 1 ml of blood loss at this time point. The fluid volumes that were administered at this time point were likened to the recommended conventional shock dose rates for cats (Broadstone 1999; Rozanski & Rondeau 2002), but less than the frequently recommended ratios. These findings have serious clinical implications because if the intravascular compartment is resuscitated using these conventional liberal resuscitation guidelines then coagulopathies can be anticipated. We speculate that a conservative approach to fluid

administration after haemorrhage aimed specifically to improve cardiovascular function and tissue oxygenation would not result in coagulopathies. Furthermore, the administrations of a limited fluid volume resuscitation protocol where hypertonic saline (2 ml kg^{-1}) alone or in combination with a hydroxyethyl starch (2 ml kg^{-1}) are administered after an initial isotonic crystalloid bolus (15-20 ml kg^{-1}) may be an alternative therapy for cats unresponsive to conventional fluid therapy protocols (Giudice et al. 2018). Our cats received very liberal fluid volumes after severe haemorrhage and despite this, they had normal coagulation and haematology profiles the day after treatments. Therefore, we speculate that healthy cats have adequate fluid tolerance and, unexpectedly, do not require emergency interventions (diuretic treatment for example) to normalise their total body water and haematocrit.

The study had notable limitations. Blood sampling method used during the health check (needle and syringe) was different from that used during subsequent time points (aspiration from a catheter), which is not ideal (Flatland et al. 2014). However, there were no differences in the tests of coagulation during health check and the day after treatments suggesting that the sampling technique did not cause deviations in outcomes of coagulation assays. A complete investigation into the pathophysiology of the derangements (platelet function analysis, factor concentration determination, etc.) was not conducted because of study budget constraints. Therefore, we cannot state with confidence whether other contributing factors played a role in the coagulopathies other than haemodilution.

5.6 Conclusions

Coagulopathies consistent with hypocoagulability were detected during the resuscitation phase in anaesthetised cats after haemorrhage. There were no differences in the coagulopathies between cats that received lactated Ringer's solution (60 mL kg^{-1} for 2 hours) and Voluven fluid (20 mL kg^{-1} for 2 hours) administration. Despite identifying coagulopathies, further research is required to identify the mechanisms and pathophysiology responsible for these derangements. Caution is advised during fluid resuscitation in cats that have undergone haemorrhage whereby more than 30% of their blood volume has been lost, especially in the light of the fact that coagulopathies were detected at the conventional fluid rates used for resuscitation with lactated Ringer's solution and Voluven in the present study.

CHAPTER 6

General conclusions

The series of investigations that constitute the present thesis focused on determining the physiological responses to an acute severe haemorrhage and responses to fluid administration to treat the consequent haemorrhage-induced hypovolaemic hypotension. The overall objective was to provide clinically relevant and practical management tools and insight into the treatment of acute severe controlled haemorrhage by fluid resuscitation during the peri-operative period in anaesthetised cats. The present series of studies describes novel tools to guide fluid therapy in cats in the form of a scoring system for acute haemorrhage and volume-endpoint biomarkers that could also be useful outside of the peri-anaesthetic period. The evidence-based information enhances the understanding of how cats respond to severe haemorrhage treated by large volume resuscitation.

6.1 Physiological effects of severe haemorrhage in anaesthetised cats

The physiological responses to acute haemorrhage in anaesthetised cats demonstrated shifts in some of the measured variables immediately after haemorrhage compared to values obtained before haemorrhage. The magnitude of change in the pre- and post-haemorrhage measured values was different for mild (< 10 ml kg^{-1}) and severe (> 22 mL kg^{-1}) haemorrhage. We found that HR and Ht increased while arterial blood pressure (SAP, DAP and MAP), $PE'CO_2$, serum Alb, and HCO_3(act) decreased; these were the most significant variables with high Cohen's *d* values. However, examining the change in a single variable was not a good indicator for quantifying the volume of acute blood loss because, during anaesthesia, there are many confounding factors related to drug administration and surgical events that could alter these variables. To mitigate these confounding factors, we found that four ratios derived from these variables allowed for a highly sensitive and specific scoring system that could be used to quantify acute blood loss. The four ratios were 1) HR:SAP (the shock index), 2) HR:$PE´CO_2$, 3) serum Alb:Ht, and 4) HR:HCO_3(act). These ratios constitute the proposed scoring system which we named the Cat Acute Bleeding Scoring System (CABSS). The CABSS provides a novel tool to assist in the quantification of acute blood loss during the peri-anaesthetic period in cats. Various scoring systems are already in use in human medicine, especially during trauma stabilisation

and surgical procedures where the risk for intraoperative haemorrhage and massive blood transfusion is high.

Intraoperative scoring systems to assess risk of intraoperative bleeding or mortality are more scantly reported and are focused on specific organ system related haemorrhage events compared to generalised pre-hospital trauma scoring systems (Pons et al. 1985; Copeland et al. 1991; Tromp Meesters et al. 1994; Ghosh et al. 2002; Wise & Clark 2008). Regardless of this observation, two major outcomes are evident when examining these scoring systems: 1) they make use of very similar variables despite having different cut-off values, and 2) they incorporate more than one variable. Furthermore, the scoring systems are designed with a specific intention or purpose, either to quantify haemorrhage, or to assess the risk for major transfusions, or to stratify patients into different risk categories, or to predict hospital stay outcome. Very few scoring systems are interchangeable where they can be used for more than their intended use. The CABSS scoring system is unique in its use of variables and purpose compared to the other scoring systems that were evaluated for this study.

The variables used in development of the CABSS were placed into ratios of variables whose direction of change after haemorrhage were opposite. For example, the SI is a ratio of HR to SAP whereby the HR is expected to increase and the SAP is expected to decrease during hypovolaemia. However, SI cannot be used alone because the relationship between blood loss volume and clinical signs (HR, SAP, f_R) is not always reliable (Pacagnella et al. 2013). Therefore, three other ratios were added to the CABSS to improve the confidence of detecting and quantifying haemorrhage. These variables are expected to decrease [SAP, $PE'CO_2$, serum Alb, HCO_3(act)] or increase (HR, Ht) reliably in response to haemorrhage-induced hypovolaemia. Furthermore, the variables that are required for the ratios are easily measurable in most veterinary practices. Therefore, the more ratios that are calculated in the CABSS, the more confident the veterinarian can be to detect and quantify the blood loss. We speculate further, based on clinical experience, that there are very few other causes that would reliably increase or decrease the variables rapidly during the peri-anaesthetic period. Furthermore, the CABSS is diverse as it incorporates clinical variables (HR, SAP) and blood variables [serum Alb, Ht, HCO_3(act)], an important characteristic of a dependable scoring system, as demonstrated by the scoring systems used in human medicine (section '1.2.4 Scoring systems used to assess acute haemorrhage'). The main purpose and intention of the CABSS are to detect and quantify haemorrhage

in anaesthetised adult cats. Furthermore, the CABSS could be used to stratify haemorrhage into different risk categories in cats.

6.2 Physiological effects of rapid fluid infusion for the treatment of haemorrhage-induced hypovolaemic hypotension in anaesthetised cats

The choice of fluid to administer to resuscitate the intravascular compartment for the treatment of hypovolaemia has been a subject of debate for decades (Cazzolli & Prittie 2015). The volume to be administered was thought to be as easy as administering a 'shock dose' or at a ratio to the blood volume lost (Davis et al. 2013). However, based on the evidence in this thesis, perhaps both of these questions still require further attention in cats. We administered a fixed volume of fluid over a set time interval to replace blood loss using the ratio to the blood volume protocol. The estimated ratios that were selected were 4:1 and 1.5:1 for lactated Ringer's solution and tetrastarch 6% 130/0.4, respectively. These ratios were specifically chosen to create a fluid overload to be able to determine meaningful endpoints of administration. We identified fluid overload in the cats, regardless of the fluid that was administered. The fluid overload allowed the investigation of a novel set of endpoints to administering fluids during the resuscitation phase of the fluid therapy which were termed volume-endpoints.

The selection of fluid type for resuscitation after severe haemorrhage did not really matter in the cats. Both fluids, if dosed correctly, could have restored the intravascular compartment with enough volume to function adequately by restoring cardiovascular and oxygenation biomarkers of resuscitation. This finding is not surprising because it has been known for a long time that the best treatment for hypovolaemia is to restore the circulating volume quickly, provided the haemorrhage has been controlled (Rudloff & Kirby 2001; Driessen & Brainard 2006). The 6% tetrastarch 130/0.4 solution demonstrated a longer therapeutic time in the present studies reaffirming that colloids are a reasonable choice for long-term volume resuscitation (Glover et al. 2014; Adamik & Yozova 2019). The traditional shock dose or ratio-based approaches to deciding how much fluid to administer needs further interrogation as iatrogenic fluid overload has been reported to occur even when administering fluids in small incremental doses over time to effect or to a predetermined targeted resuscitation endpoint (Prittie 2006). The traditional resuscitation endpoints focus on restoring cardiovascular physiological function to ensure optimal tissue oxygenation (Rudloff & Kirby 2001; Driessen & Brainard 2006; Mensack 2008).

Therefore, the goals of administering fluids during the resuscitation phase of the fluid therapy plan are to restore the intravascular volume, arterial blood pressure and cardiac output, to restore global perfusion and oxygenation. However, using these traditional resuscitation endpoints has limitations, namely: 1) lack of consensus of how much volume should be administered before alternative treatments are instituted, 2) lack of consensus on which single biomarker to use, 3) poor performance as early biomarkers for fluid overload, and 4) additional costs to the client (Rudloff & Kirby 2001; Driessen & Brainard 2006; Mensack 2008; Thomovsky et al. 2016; Ostroski et al. 2017). Considering these limitations, and the risk and high odds of causing a fluid overload, we identified volume-endpoints that are easy to determine and cost-effective. Ideally, these volume-endpoints should identify a point where additional administration of fluids begins to disrupt normal physiological function, thus compromising convalescence. We demonstrated that the Ht, serum Alb, total magnesium, chloride to sodium ratio, sodium chloride differences were variables that could potentially determine a volume-endpoint. However, the two strongest biomarkers were total magnesium and the chloride to sodium ratio. The volume-endpoints indicated an initial resuscitation volume of 30 ml kg^{-1} or 10 ml kg^{-1} for lactated Ringer's solution or 6% tetrastarch 130/0.4, respectively, over 15 to 20 minutes for cats that have sustained an acute controlled intraoperative haemorrhage of approximately 24 ml kg^{-1}. This volume translates into a ratio of approximately 1.25:1 and 0.4:1 for lactated Ringer's solution or 6% tetrastarch 130/0.4, respectively. These observed resuscitation volumes (or ratios) are less than previously recommended but in agreement with new emerging evidence in human medicine (Dunser et al. 2013; Hahn 2013; Lira & Pinsky 2014; Laszlo et al. 2017; Fodor et al. 2019). At these newly proposed initial volumes for resuscitation, there were no clinically relevant acid-base or coagulation related derangements, which implies preservation of normal physiological function. A long-held view is that cats are a fluid-sensitive species and that there is risk of morbidity or mortality associated with fluid administration during anaesthesia (Brodbelt et al. 2007; Thomovsky et al. 2016). Perhaps the aged guidelines, extrapolating fluid therapy plans from other species, incidentally have caused fluid overload in cats.

Are cats uniquely adapted in that they do not require traditional approaches to volume resuscitation? We question this sentiment because we aimed to cause fluid overload by administering fluid at volumes which far exceed the known limits of causing fluid overload in cats, but not all cats in the present study demonstrated fluid overload. Our criteria to define fluid overload were not ambiguous in that the signs used for its assessment [facial and limb anasarca and nasal oedema (demonstrated as clear fluid

discharging from the nose)] were easily observable. If cats were fluid-sensitive, then one could speculate that they would struggle to cope with the excessive fluids for a few days. However, in the present study, nasal discharges dried up within 3 hours and the cats were able to breathe comfortably thereafter. Facial and limb oedema were not clinically detectable six hours after recovery and all assessed physiological parameters had returned to within normal reference intervals for cats by the next day. Furthermore, we did not administer any diuretics to hasten fluid shedding. The cats were observed for pain and life-threatening disorders related to fluid overload (such as lung oedema, haemorrhage from carotid artery catheterisation site) during the recovery period, but no supportive intervention was needed in any of the cats for any of the treatments. After receiving a gross fluid overdose, all cats managed to excrete the excess fluid within hours and returned to normal function within 24 hours. These observations suggest that cats are not fluid-sensitive, but rather physiologically adapted to require much less fluid than commonly thought. Perhaps our proposed volume-endpoint biomarkers, the chloride to sodium ratio and magnesium concentration, would solve the question of how much fluid a cat should receive for resuscitation.

6.3 Future recommendations

The thesis introduces two novel ideas, namely the Cat Acute Bleeding Scoring System (CABSS) and the electrolyte-based volume-endpoints (specifically the chloride to sodium ratio) to guide administration of fluids in cats. These ideas emanated from a controlled haemorrhage model in cats under general anaesthesia, which is a likely clinical situation that anaesthesiologists would be facing. However, there is a need to further evaluate these ideas in different clinical scenarios, such as in conscious cats that are critically ill or after trauma, to assess whether they are indeed valid. Furthermore, the CABSS intended use is not only to quantify intraoperative haemorrhage, but to trigger a transfusion, or as a stratification tool for risk in haemorrhagic cats or as a predictor for hospitalised case outcomes.

The volume-endpoints allowed the determination of initial volumes of lactated Ringer's solution and 6% tetrastarch 130/0.4 to administer if severe haemorrhage is suspected or confirmed in cats. These evidence-based recommendations required further evaluation in other clinical scenarios. Cats that are injured or ill could have increased oxygen requirements and therefore preventing fluid overload would be paramount to successful treatment. Iatrogenic fluid overload causes tissue lung oedema and

decreases the haematocrit (or haemoglobin concentration) possibly compromising tissue oxygenation. Furthermore, the administration of fluids for restoration of the circulating volume could increase cardiac output despite the expected decrease in haematocrit creating an isovolumic haemodilution state. The latter is known to improve microvascular perfusion which could be beneficial. Further research is required to confirm this speculation. Fluid therapy, in general, needs to be more thoroughly investigated in cats.

In the present study series, we did not identify clinically relevant acid-base derangements. The most clinically relevant derangement was related to coagulation, with evidence of hypocoagulopathy after severe haemorrhage and liberal fluid resuscitation. However, we don't know whether these findings would be relevant to trauma- or critically-ill cases. We did not investigate the effects of severe haemorrhage and liberal fluid resuscitation on the glycocalyx and the inflammatory cascade. Further investigations should integrate the coagulation and inflammatory cascades during haemorrhage and fluid resuscitation.

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Animal Ethics Certificates

A.1 Certificate University of Pretoria (v006-15)

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Physiological effects of rapid intravenous infusion of fluids for the treatment of hypovolaemic hypotension in anaesthetised cats**
PROJECT NUMBER	**V006-15**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr Gareth Zeiler**

STUDENT NUMBER (where applicable)	**UP_21039993**
DISSERTATION/THESIS SUBMITTED FOR	**PhD**

ANIMAL SPECIES	**Feline (Cats)**	
NUMBER OF ANIMALS	**8**	
Approval period to use animals for research/testing purposes		**1 May 2015-1 May 2016**
SUPERVISOR	**Prof. TB Dzikiti**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date	20 April 2015
CHAIRMAN: UP Animal Ethics Committee	Signature	

S4285-15

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Animal Ethics Committee

Extension No. 1

PROJECT TITLE	**Physiological effects of rapid intravenous infusion of fluids for the treatment of hypovolaemic hypotension in anaesthetised cats**
PROJECT NUMBER	**V006-15**
RESEARCHER/PRINCIPAL INVESTIGATOR	**Dr Gareth Zeiler**

STUDENT NUMBER (where applicable)	**UP_21039993**
DISSERTATION/THESIS SUBMITTED FOR	**PhD**

ANIMAL SPECIES	**Feline (Cats)**	
NUMBER OF ANIMALS	**8**	
Approval period to use animals for research/testing purposes		**November 2016-November 2017**
SUPERVISOR	**Prof. TB Dzikiti**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 22 November 2016
CHAIRMAN: UP Animal Ethics Committee	Signature

S4285-15

A.2 Certificate University of Witwatersrand (2017-10-68-C-AREC)

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

ANIMAL ETHICS SCREENING COMMITTEE (AESC)

Registration number: AREC0101210-002

Solomon Mahlangu House
10th Floor, Room 10004
Jorissen Street
Braamfontein, Johannesburg

11 December 2017

To whom it may concern,

Re: Waiver from Animal Ethics Screening Committee of the University of the Witwatersrand

This letter serves to confirm that Associate Professor Gareth Zeiler registered for a higher degree (PhD-School of Physiology, University of the Witwatersrand) has been granted a waiver for a full animal ethics clearance application by the animal research ethics committee (AREC) of the University of the Witwatersrand. The waiver is for a study titled "The physiological effects of rapid intravenous infusion of fluids for the treatment of hypovolaemic hypotension in anaesthetized cats". The study is supervised by Professor Andrea Fuller (University of the Witwatersrand), Professor Leith Meyer (University of Pretoria) and Professor Brighton Dzikiti (West Indies).

Reasons: The study has been reviewed and approved by the animal ethics committee of the University of Pretoria (Reference V006-15). The study will be undertaken at the University of Pretoria.

Condition: The student and supervisors should ensure compliance with all terms and conditions set by the animal ethics committee of the University of Pretoria. Progress reports should be submitted annually to the AREC (University of the Witwatersrand). Any changes made to the current approved protocol should be communicated to the AREC.

Should you require any further information, do not hesitate to contact me.
Yours sincerely,

Kennedy Erlwanger
(Chairman: Animal Ethics Screening Committee, University of the Witwatersrand)

Reference: G Zeiler-2017-10-68-C-AREC Waiver 2017

Assoc Prof Kennedy H. Erlwanger
School of Physiology
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Blood volumes and data collection during experimental procedures

A.3 Planned blood volumes drawn for a 4 kg cat

The following anticipated volumes of blood will be collected for a 4 kg cat over a 24 hour period:

Event	Volume withdrawn	Accumulative volume	Volume per kilogram	Per cent blood loss
Volume of Blood Loss				
Pre-treatment Phase				
EDTA, Serum, Citrate	4 mL	4 mL	1 mL kg^{-1}	1.56%
Venous Blood gas	1 mL	5 mL	1.25 mL kg^{-1}	1.95%
Anaesthesia and Instrumentation Phase				
EDTA, Serum, Citrate	4 mL	9 mL	2.25 mL kg^{-1}	3.52%
Arterial Blood gas	1 mL	10 mL	2.5 mL kg^{-1}	3.91%
Blood loss during cannulation	5 mL	15 mL	3.75 mL kg^{-1}	5.86%
Treatment Phase				
Controlled Haemorrhage Stage				
EDTA, Serum, Citrate	12 mL	27 mL	6.75 mL kg^{-1}	10.55%
Arterial Blood gas	3 mL	30 mL	7.5 mL kg^{-1}	11.72%
Blood collection	30 mL	60 mL	15 mL kg^{-1}	23.43%
Treatment Stage				
EDTA, Serum, Citrate	9 mL	69 mL	17.25 mL kg^{-1}	26.95%
Arterial Blood gas	3 mL	72 mL	18 mL kg^{-1}	28.13%
Post-treatment Phase				
EDTA, Serum, Citrate	9 mL	81 mL	20.25 mL kg^{-1}	31.64%
Arterial Blood gas	3 mL	84 mL	21 mL kg^{-1}	32.81%
	TOTAL	**84 mL**	**21 mL kg^{-1}**	**32.81%**
Note: Assumption based on a 4 kg cat that has a circulating blood volume of 256 mL. This table highlights the blood loss and not the volume of fluid replaced to maintain the circulating volume and mean arterial blood pressure administered during the treatment phase.				
Fluid Resuscitation (Volume Replacement)				
Treatment Phase				
Treatment Stage				
Effect of circulating volume replacement with resuscitation fluid	30 mL	54 mL	13.5 mL kg^{-1}	21.10%
	TOTAL	**54 mL**	**13.5 mL kg^{-1}**	**21.10%**
Note: The volume of resuscitation fluid will be unknown. However, the clinical effect is expected to restore the circulating volume to pre-controlled haemorrhage phase volume.				

The blood volume loss during the controlled haemorrhage stage of the treatment phase will be replaced by the different fluids administered during the treatment stage of the treatment phase of the study. This replacement volume will be calculated once the mean arterial blood pressure has been restored to the pre-treatment value. It is expected that the fluid used to resuscitate the volume depleted cat will replace the anticipated 30 mL withdrawn during the controlled haemorrhage stage. Thus the volume of blood lost to cannula placement and blood testing is estimated to be 54 mL (84 mL – 30 mL = 54 mL). Healthy cats undergoing blood donation may donate 10 to 12 mL kg^{-1} safely (estimated 18 to 20% of circulating blood volume). Once the final 24 hour data collection point has been reached, the cat will receive the withdrawn blood obtained during the simulated haemorrhage.

A.4 Planned data collection during a treatment cycle

Data type	Sample	Frequency	Note
Pre-treatment phase			
Basic clinical examination	Temperature; pulse; respiration	Once on day before the treatment	Ensure cat is in a healthy, physiologically normal state
Venous blood	EDTA and serum tube (1 mL each)	Once on day before the treatment	To determine baseline pre-anaesthetic cytology (complete blood count) and biochemistry (albumin, globulin, creatinine, urea, magnesium, phosphate levels) are within normal limits
Venous blood	Citrate tube (2 mL)	Once on day before the treatment	Thromboelastography, partial thromboplastin, prothrombin time and antithrombin
Venous Blood gas	1 mL sample	Once on day before the treatment	For electrolytes
Anaesthesia and instrumentation phase			
Multiparameter physiological data	ECG; respiratory and heart rate; systolic, mean and diastolic arterial blood pressure; central venous pressure; end-tidal carbon dioxide and isoflurane; fractional inspired concentration of oxygen; peripheral arterial haemoglobin saturation; oesophageal temperature (Datex multiparameter monitor); muscle oxygen tension; cardiac output	Two readings (5 minutes apart) for pre-treatment (baseline; $T0_{haemorrhage}$) readings; then at 5 to 15 minute intervals depending on the parameter	To use purpose designed anaesthetic monitoring sheet to capture this data. A critical event data sheet will capture key data sets of data at pre-treatment (baseline), controlled haemorrhage stage ($T0_{haemorrhage}$; mid-haemorrhage, end-haemorrhage); treatment stage ($T0_{treatment}$, every 15 minutes during treatment until 120 minutes).
Arterial blood gas	1 mL sample from femoral cannula into lithium heparinised syringe	Once for pre-treatment (baseline; $T0_{haemorrhage}$)	Measure pH, partial pressure of oxygen and carbon dioxide, sodium, potassium, calcium and bicarbonate. To run haematocrit, lactate and glucose on this sample.

Blood sample	1 mL sample from femoral cannula into serum tube	Once for pre-treatment (baseline; $T0_{haemorrhage}$)	Measure albumin, globulin, creatinine, urea, magnesium, phosphate levels
Blood sample	1 mL sample from femoral cannula into EDTA tube	Once for pre-treatment (baseline; $T0_{haemorrhage}$)	Complete blood count
Blood sample	2 mL sample from femoral cannula into citrate tube	Once for pre-treatment (baseline; $T0_{haemorrhage}$)	Thromboelastography, partial thromboplastin, prothrombin time and antithrombin
Thoracic radiograph	Lateral and ventro-dorsal thoracic radiographs	Once for pre-treatment (baseline; $T0_{haemorrhage}$)	Determine normal radiographic image
Treatment phase			
Controlled haemorrhage stage			
Arterial blood gas	1 mL sample from femoral cannula into lithium heparinised syringe	Once every 6 mL of blood withdrawn. The 1 mL is a part of the 6 mL volume (4 mL + 2 mL = 6 mL; see serum sample below)	Measure pH, partial pressure of oxygen and carbon dioxide, sodium, potassium, calcium and bicarbonate. To run haematocrit, lactate and glucose on this sample.
Blood sample	1 mL sample from femoral cannula into serum tube	Once every 6 mL of blood withdrawn; see arterial blood gas comment above.	Measure albumin, globulin, creatinine, urea, magnesium, phosphate levels
Blood sample	2 mL sample from femoral cannula into citrate tube	Once at end of controlled haemorrhage	Thromboelastography, partial thromboplastin, prothrombin time and antithrombin
Blood sample	1 mL sample from femoral cannula into EDTA tube	Once at end of controlled haemorrhage	Complete blood count
Treatment stage			
Arterial blood gas	1 mL sample from femoral cannula into lithium heparinised syringe	At beginning of fluid infusion ($T0_{treatment}$), then at 60 and 120 minutes.	Measure pH, partial pressure of oxygen and carbon dioxide, sodium, potassium, calcium and bicarbonate. To run haematocrit, lactate and glucose on this sample.
Blood sample	1 mL sample from femoral cannula into serum tube	At beginning of fluid infusion ($T0_{treatment}$), then at 60 and 120 minutes.	Measure albumin, globulin, creatinine, urea, magnesium, phosphate levels

Blood sample	2 mL sample from femoral cannula into citrate tube	At 60 and 120 minutes of treatment	Thromboelastography, partial thromboplastin, prothrombin time and antithrombin
Blood sample	1 mL sample from femoral cannula into EDTA tube	At 60 and 120 minutes of treatment	Complete blood count
Thoracic radiograph	Lateral and ventro-dorsal thoracic radiographs	Once for post-treatment	Determine early volume overload
Post-treatment phase			
Arterial blood gas	1 mL sample from femoral cannula into lithium heparinised syringe	At 2, 6 and 24 hours post recovery	Measure pH, partial pressure of oxygen and carbon dioxide, sodium, potassium, calcium and bicarbonate. To run haematocrit, lactate and glucose on this sample.
Blood sample	1 mL sample from femoral cannula into serum tube	At 2, 6 and 24 hours post recovery	Measure albumin, globulin, creatinine, urea, magnesium, phosphate levels
Blood sample	2 mL sample from femoral cannula into citrate tube	At 2, 6 and 24 hours post recovery	Thromboelastography, partial thromboplastin, prothrombin time and antithrombin
Blood sample	1 mL sample from femoral cannula into EDTA tube	At 2, 6 and 24 hours post recovery	Complete blood count
Thoracic radiograph	Lateral and ventro-dorsal thoracic radiographs (physical restraint)	24 hours	Determine late volume overload

A.5 Randomisation and study dates

Cat	Treatment period 1 30/05/2017 to 08/06/2017	Treatment period 2 07/08/2017 to 23/08/2017	Treatment period 3 23/10/2017 09/11/2017
Happy	Control	Voluven	LRS
Paige	LRS	Control	Voluven
Rae	Voluven	Control	LRS
Ralph	LRS	Voluven	Control
Sylvester	Voluven	LRS	Control
Toby	Control	LRS	Voluven

Rescue interventions

A.6 Planned rescue interventions during the treatment

Anaesthesia and instrumentation phase

Potential complications:

1. Induction apnoea for more than 1 minute.
2. Hypoxaemia detected on arterial blood gas (regarded as an arterial partial pressure of oxygen less than 60 mmHg) or pulse oximetry (peripheral haemoglobin saturation of < 90%) for more than 2 minutes.
3. Hypotension where baseline mean arterial blood pressure readings are < 60 mmHg for more than 10 minutes.

Rescue intervention:

1. Intermittent positive pressure ventilation making use of the reservoir bag attached to the Mapleson D breathing system. A rate of 5 breaths minute^{-1} until a rise in chest is observed will be used until spontaneous respiration begins.
2. The fractional inspired concentration of oxygen will be increased by 10% increments until hypoxaemia is resolved. If hypoventilation is suspected to be the cause (end-tidal CO_2 > 40 mmHg) then the end-tidal isoflurane will be decreased by 0.25% every 5 minutes until regular spontaneous respiratory pattern and an end-tidal CO_2 reading of ≤ 40 mmHg are achieved.
3. If the isoflurane overdosage is suspected to be the cause of the hypotension then the end-tidal isoflurane concentration will be decreased until a stable normotensive state is achieved. If the heart rate is less than 80 beats minute^{-1} then bradycardia may be the cause of hypotension. This will be treated with glycopyrrolate (0.005 mg kg^{-1}) to increase the heart rate. Pre-anaesthetic starvation and withholding water may contribute to a mild hypovolaemia; a single bolus of lactated Ringer's solution (5 mL kg^{-1} over 15 minutes) will be administered to correct the intravascular volume. If this does not resolve, the cat could be recovered and reintroduced to the study after a minimum 48 hour cage rest.

Treatment phase

Controlled haemorrhage stage

Potential complications:

1. Severe hypotension where mean arterial blood pressure is < 40 mmHg.
2. Severe hypotensive state associated with bradycardia (heart rate < 75 beats minute^{-1}).
3. Cardiovascular collapse and asystole.

Rescue intervention:

1. Begin the assigned resuscitation strategy without delay, if after 10 minutes the patient is still in a severe hypotensive state then to bolus an amount of the fluid used during the resuscitation at the following recommended rates: Lactated Ringer's solution (20 mL kg^{-1}); Voluven (5 mL kg^{-1}); Oxyglobin (4 mL kg^{-1}); Blood (4 mL kg^{-1}) all administered over a 20 minute period, or until mean arterial pressure rises above 45 mmHg. Once a minimum mean arterial blood pressure of 45 mmHg has been achieved then the fluid resuscitation strategy will continue at the pre-defined treatment stage rate. If severe hypotension persistent then to decrease the end-tidal isoflurane concentration by 0.25% every five minutes until blood pressure improves.

2. Decrease the end-tidal isoflurane concentration. Administer an anticholinergic as follows: atropine (0.03 mg kg^{-1}) using the intravenous route.

3. Stop delivery of isoflurane, flush breathing system with 100% oxygen. Begin cerebral cardiorespiratory resuscitation by veterinary anaesthetist. Low dose adrenaline (0.25 mL $10kg^{-1}$ of the 1 mg mL^{-1} solution) to be administered at 3 minute intervals. Administer their blood back via rapid manual syringe transfusion. A blood micro-filter will be attached directly to the indwelling intravenous cannula prior to blood transfusion. Such a cat will be excluded from the rest of the study and replaced by another cat to continue the treatments remaining.

Treatment stage

Potential complications:

1. Profound haemodilution where packed cell volume decreases to < 0.2 L L^{-1}.
2. Failure to achieve a return to pre-treatment mean arterial blood pressure state within 120 minutes.
3. Arrhythmias
4. Hypocalcaemia (ionised Ca^{2+} < 1.1 mmol L^{-1}), especially post blood transfusion.

Rescue intervention:

1. Stop fluid resuscitation. Consider administering blood at 2 mL kg^{-1} to increase packed cell volume by 0.01 L L^{-1} (1%), until a packed cell volume of 0.2 L L^{-1} is reached.
2. Consider administering boluses during recovery (treatment effect considered not adequate to restore intravascular volume in cat). Rescue intervention bolus will be based on the treatment allocation. Boluses are as follows: Lactated Ringer's solution (20 mL kg^{-1}); Voluven (5 mL kg^{-1}); Oxyglobin (4 mL kg^{-1}). If the cat is undergoing the treatment LB then they will receive Voluven as their rescue intervention. A partial or complete volume blood transfusion may also be considered, depending on the clinical presentation of the cat prior to rescue intervention.
3. Lidocaine, atropine and glycopyrrolate will be available to treat any common arrhythmia that may be detected during the resuscitation phase. Intervention will be instituted by a veterinary anaesthetist on a case to case basis.
4. Hypocalcaemia will be treated with intermittent intravenous boluses (0.25 mL kg^{-1}) of calcium gluconate 10% solution.

Post-treatment phase

Potential complications:

1. Delayed recovery from general anaesthesia (expected to recover within 30 minutes).
2. Muscle fasciculation and/or seizures.
3. Evidence of volume overload (pleural effusion, pulmonary oedema)
4. Evidence of pain based on clinical judgement of a veterinary anaesthetist.
5. Persistent hypotension post recovery and initial fluid bolus intervention described in "treatment stage".
6. Blood transfusion reaction (restlessness, vocalisation, tachycardia, bradycardia, tachypnoea, hypotension, hypertension, pyrexia, urticaria or emesis).

Rescue intervention:

1. Conduct clinical examination and correct any typical physiological deficits such as hypothermia experienced during general anaesthesia. Points 2 to 5 may also be causes of delayed recovery and will be treated as explained in the next points.
2. Test for possible hypoglycaemia and/or hypocalcaemia. Hypoglycaemia will be treated with 0.5 to 1 mg kg^{-1} boluses of dextrose 10% administered intravenously. Hypocalcaemia will be treated with intermittent intravenous boluses (0.25 mL kg^{-1}) of calcium gluconate 10% solution.
3. Cats suffering from volume overload will be treated with boluses of furosemide (0.5 to 1.0 mg kg^{-1}) via the intravenous route. Supportive measurements and monitoring of urine output frequency (subjective litter box inspection) will be based on case presentation. Hypoxaemia and/or cyanosis may require oxygen cage therapy until the lungs are able to function within normal limits.
4. Rescue analgesia includes repeated boluses of buprenorphine (0.02 mg kg^{-1}) every 8 hours and/or a bolus of meloxicam (0.2 mg kg^{-1}), provided the cat is in a normotensive state.
5. If the cat is still considered to be hypovolaemic (anuria, delayed capillary refill time) then the blood will be transfused back to the cat at an appropriate rate before the 24 hours end point is reached. If the cat is considered normovolaemic and is otherwise physiologically stable, a catecholamine constant rate infusion may be considered (dobutamine, dopamine) or a bolus of ephedrine (0.1 mg kg^{-1}) will be administered.
6. Transfusion reactions will be treated symptomatically. A single bolus of dexamethasone (0.5 mg kg^{-1}) will be administered subcutaneously if signs of transfusion reactions are noticed. If it is suspected to be due to a high rate of transfusion, then the rate will be decreased.

Other possible complications requiring rescue intervention not mentioned in this protocol will be evaluated and treated on a case by case approach. Appropriate intervention will be sought after to ensure that the cats remain in a healthy, physiologically normal, stress-free (within reason) and pain-free state at the UPBRC

Proof of published article

A.7 Development of a severity scoring system for acute haemorrhage in anaesthetised domestic cats: The CABSS score

Decision Letter (VAA-19-0123.R2)

From: VAA.EIC.19@gmail.com

To: gareth.zeiler@up.ac.za

CC: kenvet@usa.net

Subject: Veterinary Anaesthesia and Analgesia - Decision on Manuscript VAA-19-0123.R2

Body: 7th November 2019,

Dear Dr. Zeiler, Dear Gareth,

It is a pleasure to accept your manuscript, subject to editing, titled "Development of a severity scoring system for acute haemorrhage in anaesthetised domestic cats: The CABSS score" for publication in Veterinary Anaesthesia and Analgesia (VAA).

On behalf of the Editors of VAA, I would like to take this opportunity to thank you for this very interesting contribution to the journal. We look forward to your continued contributions in the future.

First Look: Please note that although the manuscript is accepted the files will now be checked to ensure that everything is ready for publication. Your paper will be edited and may be returned to you to agree or disagree (please provide reasons) with any major changes. This should ensure that authors and editors are in agreement before the manuscript is sent to production services. It is not until the editing process is complete that your files will be forwarded. To minimise publication time of your manuscript it is important that all electronic artwork is supplied to the editorial office in the correct format and resolution. I recommend that you consult the Illustration guidelines at https://www.elsevier.com/authors/author-schemas/artwork-and-media-instructions if you need advice on any aspect of preparing your artwork.

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Once your manuscript has been forwarded onto the Production Office, the Production Editor will be in contact in due course regarding the author forms and proofs of your paper.

Yours sincerely,

Rachel Bennett (Dr)
Editor, Veterinary Anaesthesia and Analgesia
VAA.EIC.19@gmail.com

Date Sent: 07-Nov-2019

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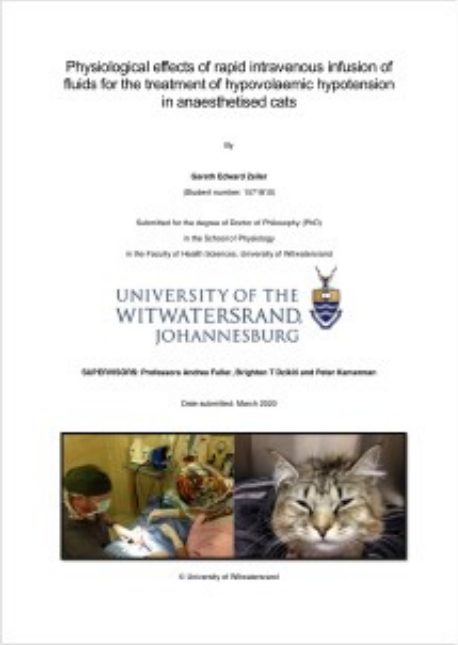
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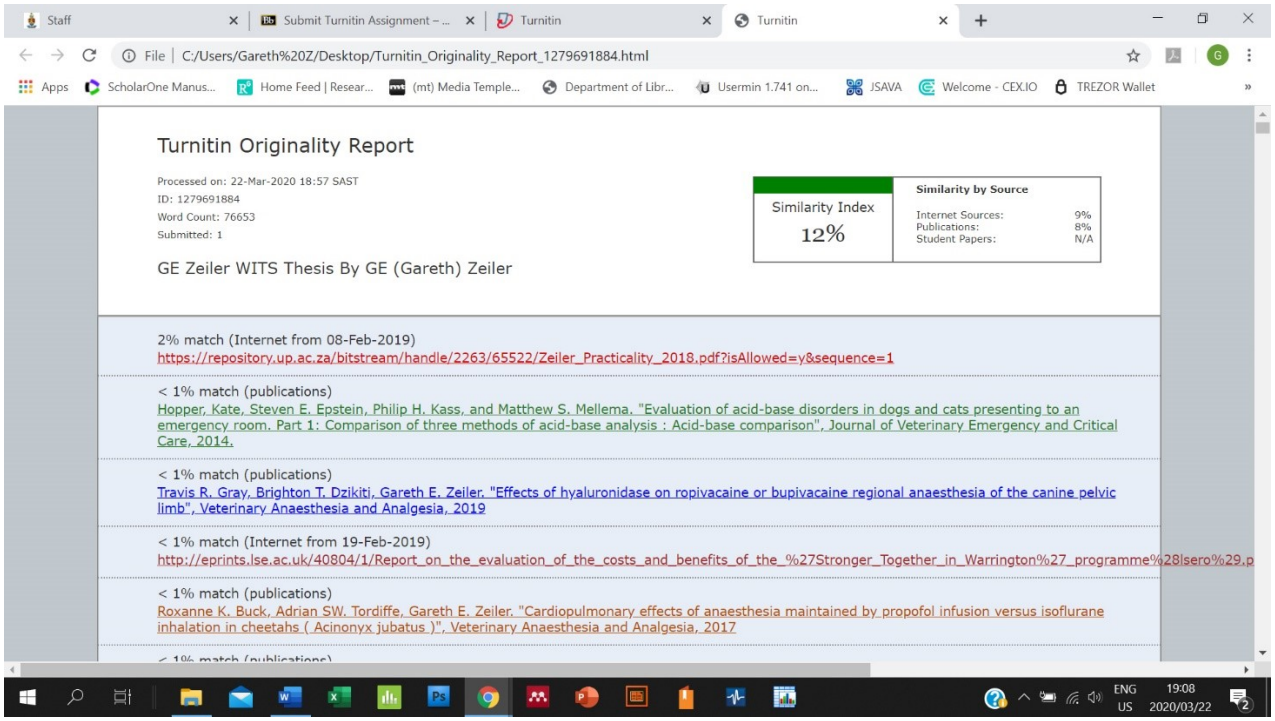


Physiological effects of rapid intravenous infusion of fluids for the treatment of hypovolaemic hypotension in anaesthetised cats

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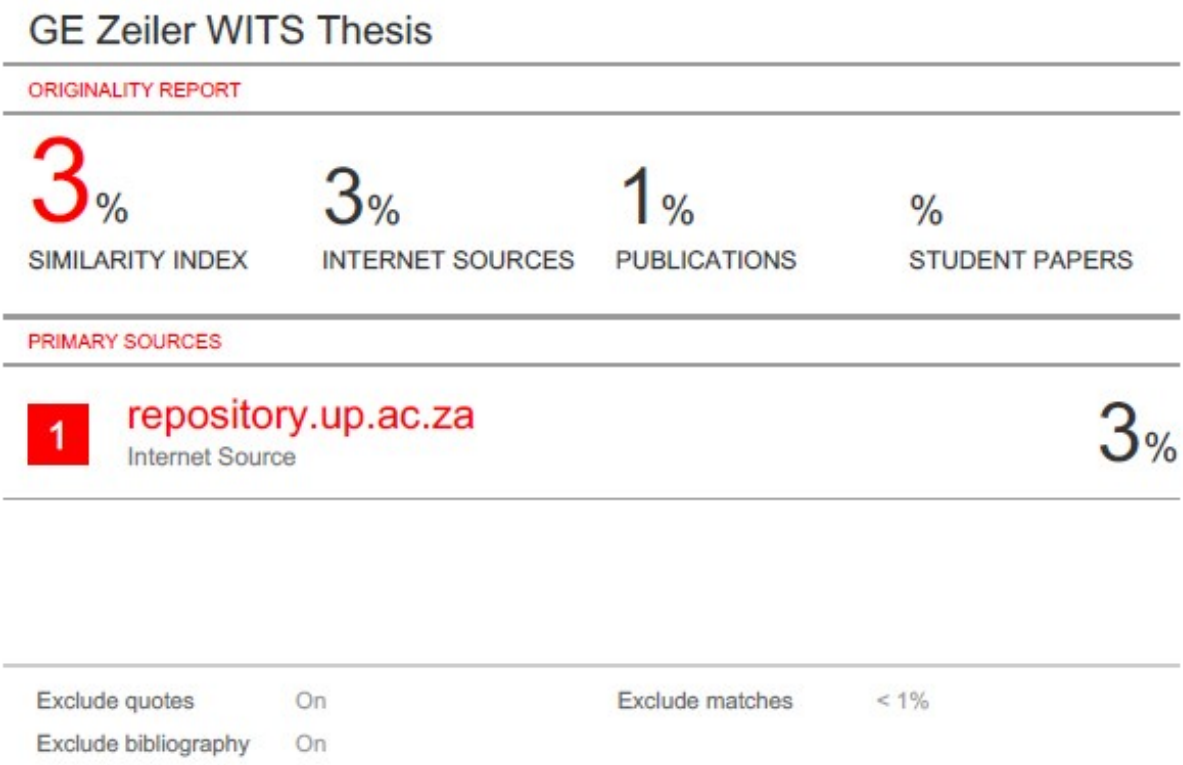
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