Assessment of volaemia using ultrasound of the heart.

Author:

Pablo M. Cortes-Sanchez: Ldo Veterinaria, GP Cert Cardiology, cert AVP, Pg cert VPS, MRCVS.

Submitted in fulfilment of the Degree of Master´s in Veterinary Medicine (MVM)

School of Veterinary medicine, College of Small Animal Clinical Sciences,

University of Glasgow

Deposited in the library in May 2021.

Abstract:

In humans, echocardiography is the standard of care in patients in shock, helping intensivists to characterize the haemodynamic disorder, to select a therapeutic approach and to monitor the response to treatment. In veterinary medicine the use of ultrasonography is limited to the assessment of the caudal vena cava (CVC), as a method of estimating volaemia. The current study aimed to explore whether echocardiographic measurements can be useful in the diagnosis and monitoring of the response to treatment in cases of suspected hypovolaemia.

The study cohort comprised of eighteen dogs presented to an out of hours service for suspicion of hypovolaemia, and the control cohort consisted of nineteen dogs presented for echocardiography that were diagnosed as not having substantial cardiac disease. Data from the physical exam (mucous membrane colour, heart rate, pulse quality, capillary refill time), systolic blood pressure (SBP), and laboratory findings (lactate concentration (LAC), total protein (TP), packed cell volume (PCV)) were recorded for the study cohort and used to decide if the patients were suspected to be hypovolaemic. Dogs from both cohorts were scanned conscious in right lateral recumbency. Ultrasonographic measurements comprised: maximal diameter of the CVC (CVC_{max}), CVC collapsibility index (CVC CI), CVC to aorta ratio (CVC/Ao), maximal dimension of left atrium to aorta ratio (LA_{major}/Ao), left ventricular internal diameter in diastole normalized for bodyweight by allometric scaling (LVIDdN), end diastolic ventricular volume index (EDVI), and stroke volume (SV) obtained from the aortic velocity- time integral. All the physical exam variables, laboratory tests and ultrasonographic measurements were measured again in the study cohort after the administration of intravenous fluid therapy.

The median time to perform the ultrasonographic protocol was 4 (3-4) minutes. The best discriminator to differentiate between cases and controls was the EDVI (P=0.047), however the specificity (42%) and sensitivity (53%) were very low. The heart rate, capillary refill time, TP, PCV, CVC_{max}, CVC/Ao, LA_{major}/Ao, LVIDdN, EDVI and SV were significantly different ($P \leq 0.05$) after treatment.

This study shows that a simplified echocardiographic protocol, that can be performed in a limited time can provide useful information about the volume status of the conscious, spontaneously breathing, clinically ill dog and can be helpful in monitoring the response to treatment.

Table of Contents

List of tables

List of figures

Acknowledgements

To Tim Parkin for all his statistical support. To Anne French for coming up with an idea to meet my two worlds: cardiology and intensive care, and all her effort and guidance during the development of the project. To Kerry Woodhouse for all her support and her insights to meet our common goal: help veterinary practitioners to do a better job.

Author's declaration

"I declare that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution."

Pablo M. Cortés-Sánchez

Abbreviations

ACVIM: American college of veterinary internal medicine

ADH: antidiuretic hormone

ALB: albumin concentration in plasma

BP: blood pressure

CO: cardiac output

CRT: capillary refill time

CVC: caudal vena cava

CVP: central venous pressure

EDVI: end diastolic ventricular volume index

HR: heart rate

IV: intravenous

LAC: lactate concentration in plasma

LA: left atrium

LV: left ventricle

LVIDdN: left ventricular internal diameter in diastole normalised by body weight.

L4chA: long axis four chamber view optimised for the left atrium.

L4chV: long axis four chamber view optimised for the left ventricle.

L5ch: long axis five chamber view

MM: mucous membrane

MMVD: myxomatous mitral valve disease

PCV: packed cell volume

PPV: pulse pressure variation

RAAS: Renin-angiotensin-aldosterone system

SBP: systolic blood pressure

Spm: short axis view at the level of the papillary muscles

SV: stroke volume

TP: total protein

VTI: velocity-time integral

2D: bidimensional mode

1. Introduction

1.1 Definitions of volaemia, hypovolaemia and hypovolaemic shock

Preserving fluid balance and maintaining homeostasis has always been one of the main targets of intensive care clinicians in human and veterinary medicine. In order to choose the appropriate therapy and monitor its effects, there are several physiological concepts that need to be clearly understood such as fluid compartments, plasma volume, and blood volume (volaemia).

1.1.1 Volaemia

Body fluids are mostly constituted of water (Reece *et al.*, 2015), which accounts for up to 60% of the total body weight in a healthy dog. Different solutes are dissolved in this water constituting the body fluids, which are contained in the different body compartments. The intracellular compartment comprises of the body fluids that are contained inside the cells, and accounts for about two thirds of the total body fluids. All the fluid located outside of the cells is considered the extracellular compartment, which is subdivided into the intravascular, interstitial, and transcellular compartments. The intravascular fluid is that contained inside the blood vessels, and usually referred to as plasma volume. The interstitial fluid is that located immediately around the capillaries and between the cells, but always outside of the cell membrane. This interstitial fluid is the major component of the extracellular fluid. The transcellular fluid is produced by specialized secreting cells. It is the smallest compartment and includes intestinal secretions, respiratory secretions, intraocular fluid, cerebrospinal fluid, and synovial fluid. All the compartments communicate with each other and equilibrate their content of fluids through a variety of mechanisms of fluid interchange across different membranes. Most disease states that cause fluid loss, initially do so in the extracellular compartment (DiBartola, 2012).

Volaemia is defined as the volume of blood circulating in the intravascular compartment (Reece *et al.*, 2015). The volume of blood is formed of two components: fluid (plasma) and cells (mostly red blood cells). Thus, a reduction in volaemia, or hypovolaemia, will occur if whole blood is lost or plasma volume is reduced by dehydration or losses to a third space.

1.1.2 Hypovolaemia

Hypovolaemia can be described as a decreased intravascular circulating fluid volume relative to the total vascular space (Pachtinger and Drobatz, 2008). Absolute hypovolaemia will occur when there is a decreased fluid volume, while relative hypovolaemia will occur when there is a normal fluid volume, but an increased vascular space, such as in the case of generalized vasodilation. Hypovolaemia will translate into a decrease in cardiac preload, which will generate a poor cardiac output and poor tissue perfusion.

In acute haemorrhage, there is a direct loss of fluid and cells (whole blood) from the intravascular compartment, leading to hypovolaemia. Dehydration occurs when the water spent by the body is superior to the intake through eating, drinking or administration of fluid therapy. The loss of water affects initially the extracellular compartment. Then, the body will try to compensate shunting fluid, mostly water, from the intracellular to the extracellular compartment. In severe dehydration, no further fluid is available to replenish the intravascular compartment (extracellular) and hypovolaemia will develop. In losses of fluid to a third space, this fluid will leave the intravascular compartment and accumulate in the interstitial space between cells, causing oedema, or in the body cavities causing effusions. A similar mechanism of that described for dehydration with compensatory shunting of fluid from the intracellular to the extracellular compartment will occur. Thus, in this scenario when hypovolaemia develops there would be a depletion of water and electrolytes in both the intra and extracellular compartments.

The body will sense the development of hypovolaemia through different receptors. Peripheral chemoreceptors in the tissues will sense a decrease in oxygen delivery secondary to the poor perfusion. Stretch receptors in the aortic arch, the carotid body and the splanchnic vessels will sense the decreased filling of these vessels. The signals sent by those receptors will trigger the activation of two main compensatory mechanisms: elevation of the sympathetic tone, with the release of epinephrine and norepinephrine, and neurohormonal response through the activation of the renin-angiotensin-aldosterone system (RAAS) and the secretion of antidiuretic hormone (ADH). The sympathetic activity will directly affect the heart and cause an increase in heart rate and cardiac contractility, thus increasing cardiac output (CO). The release of epinephrine and norepinephrine from the adrenal glands will cause the same cardiac effects as the sympathetic activity

(enhanced contractility and increased heart rate), and most importantly vascular effects with vasoconstriction, which will produce an increase in blood pressure (BP). The neurohormonal response with the activation of the RAAS and the secretion of ADH will lead to the retention of sodium and water, increasing the circulating volume, and to vasoconstriction, increasing the BP. In summary, all the compensatory mechanisms aim to restore the intravascular volume and maximize the cardiac output. When these compensatory mechanisms are overwhelmed hypovolaemic shock develops.

1.1.3 Hypovolaemic shock

Shock is defined as a defective perfusion and oxygen delivery to the tissues to the point where their minimum requirements are not met (DiBartola, 2012). If either, the requirements are pathologically increased, or the supply is short, shock will develop. Increased requirements can be seen in cases of convulsions, heat stroke, and malignant hyperthermia. Insufficient oxygen supply can be a consequence of low CO or low oxygen content of the arterial blood. These different mechanisms allow to generate a classification of shock (Ettinger *et al.*, 2016) into six main categories, outlined in Table 1.1. Clinical patients can have syndromes which borrow characteristics from different categories.

Hypovolaemic shock is the most common category seen in critical patients in veterinary medicine (Silverstein and Hopper, 2014). Thus, one of the main targets of the intensive care clinician should be to assess the volume status of the critically ill patient. Shock develops as a progressive process that can be divided into stages: compensatory, early decompensatory and decompensatory (DiBartola, 2012).

Table 1-1. Categories of shock

Shock category	**Main characteristic**	**Examples**
Hypovolaemic	Decrease effective circulating volume	Haemorrhage Severe dehydration
Cardiogenic	Decreased forward flow	Myocardial failure Arrhythmias
Obstructive	Obstruction to blood flow	Pericardial effusion Thromboembolism
Distributive	Inappropriate vascular tone	Sepsis Anaphylaxis
Hypoxaemic	Reduced content of oxygen in arterial blood	Anaemia Severe pulmonary disease
Metabolic	Impaired metabolic activity	Hypoglycaemia Toxic: cyanide

In the compensatory phase, the neurohormonal response is triggered by the stretch receptors. The CO will be increased by the compensatory mechanisms, sympathetic activity increase, RAAS activation and ADH secretion, and may reach an acceptable level, achieving compensation. However, launching all these compensatory mechanisms is energetically demanding and puts the body in a hypermetabolic state. Therefore, these compensatory mechanisms cannot be maintained indefinitely. If the intravascular volume remains inappropriate, the body runs out of energy to maintain all these compensatory mechanisms activated. Therefore, the vascular resistance starts to fall, the cardiac performance starts to deteriorate, and decompensation begins.

In the early decompensatory stage, the blood flow is redirected to the most vital organs: brain and heart, which reduces even further the oxygen delivery to the other organs. Hypoxia in the intestine can predispose to ulceration and bacterial translocation. Hypoxia in the pancreas induces the release of the myocardial depressant factor which reduces cardiac contractility and impairs the metabolism of cardiac muscle, also predisposing to the development of arrhythmias. The shunting of blood away from the kidneys reduces glomerular filtration rate and oliguria develops. The shunting of blood away from the lungs

reduces the oxygen/carbon dioxide interchange, decreasing the content of oxygen of the arterial blood, and thus, decreasing even further the oxygen that finally reaches the tissues. If no aggressive treatment is provided the lack of oxygen continues and the shock state moves to the last stage, called decompensatory stage.

In the decompensatory stage the severe and prolonged hypoxia induces a disruption in the normal regulatory mechanisms of homeostasis. A generalized vasodilation occurs, causing general circulatory failure. The sympathetic activity in the brain ceases and the heart rate and contractility fall. Eventually there is multiple organ dysfunction and death. The compensatory and early decompensatory stages can be reversed if an appropriate volaemia is restored through the administration of fluid therapy. The decompensatory stage can also be reversed, but accomplishing this reversal will require additional support, like, for instance, a blood transfusion or mechanical ventilation. Thus, the restoration of volaemia alone in the decompensatory stage will not be enough to prevent death or severe sequelae in patients who have reached this stage.

The administration of fluid therapy to correct hypovolaemia is paramount in the intensive care unit. However, not only fluid deficit, but also fluid overload can be detrimental. In human medicine, positive fluid overload correlates with increased mortality in patients suffering from sepsis (Boyd *et al.,* 2011) and in patients with respiratory distress (Rosenberg *et al.,* 2009). Although counterintuitive, fluid overload increases the risk for the development of acute kidney injury in critically ill humans (Salahuddin *et al.,* 2017). It is hypothesised that the fluid overload will cause renal interstitial oedema which will lead to increased pressure inside the renal capsule, reducing the glomerular filtration rate and leading to acute renal injury (Joannidis *et al.,* 2010). An association between fluid overload and increased mortality has been shown in dogs in intensive care units (Cavanagh *et al.,* 2016). Therefore, recognising hypovolaemia and performing an intervention of the appropriate intensity is crucial for a good outcome in the intensive care unit. Different tools are available for the assessment of volaemia. Other than the physical exam, they differ substantially between human (Cecconi *et al.,* 2014, Van der Mullen *et al.,* 2018) and veterinary medicine (Marshall *et al.,* 2016). The different approach between human and veterinary patients is due to obvious differences in the technology and staff available at most veterinary premises compared to human medicine.

1.2 Current techniques to estimate volaemia in dogs.

There are several methods available to estimate the volume status of the dog. Most of them have been adopted from human medicine. The assessment of volaemia is currently based on:

- Physical examination
- Invasive methods
- Non-invasive methods
- Laboratory measurements

1.2.1 Physical examination

The physical examination remains the main source of information for most veterinary clinicians. Even though the physical exam provides vital information, it is in many ways subjective, and many times confusing. It has been reported that clinical examination and vital signs are poorly correlated with volume status in dogs (Perel *et al.*, 1987). The main parameters to assess the volume status in the physical exam of a dog would be (Johnson, 2016): mucous membrane colour, capillary refill time (CRT), skin turgor, pulse quality and heart rate. Alterations in these parameters can reflect the activation of the compensatory mechanisms described earlier (RAAS activation, ADH secretion, and increased sympathetic activity) and raise a suspicion of fluid deficit. However, none of them can reliably estimate the blood volume status of the patient. In addition to that, the stress suffered by dogs from being in a clinical environment can trigger similar effects, elevating the heart rate and the levels of circulating catecholamines (Höglund *et al.*, 2012). The usual findings in hypovolaemic states in dogs are outlined in Table 1.2 according to the degree of severity (Boag and Hughes, 2005).

Table 1-2. Physical exam findings in hypovolaemia in dogs (Boag and Hughes 2005).

Variable	**Mild hypovolaemia**	**Moderate hypovolaemia**	**Hypovolaemic shock**
Heart rate	130-150bpm	150-170bpm	170-220bpm
Mucous membrane colour	Normal to pinker	Pale pink	Grey, white
Capillary refill time	Rapid (<1sec)	Normal (1-2secs)	Prolonged (>2secs) or absent
Pulse quality	Strong	Fair	Weak or absent

As said earlier, these changes reflect the activation of the compensatory mechanisms, and the more pronounced they are, meaning the higher the HR, the weaker the pulses, and the longer the CRT, the closer the patient is to developing hypovolaemic shock. One study investigated the usefulness of the so called "shock index", to identify dogs in shock (Porter *et al.*, 2013). The shock index is the result of dividing the HR expressed in beats per minute (bpm) by the systolic blood pressure (SBP) measured in millimetres of mercury (mmHg). They concluded that a shock index greater than one, has a good sensitivity and specificity for the diagnosis of moderate to severe shock. The reference value provided by this study for healthy dogs for the shock index was 0.37 to 1.30, which means some of the normal dogs had values over the proposed cut-off, reducing the accuracy of this index. In addition to that, they only assessed dogs with moderate to severe shock, which means there is no indication of this index for the detection of occult hypoperfusion. Occult hypoperfusion refers to a haemodynamic state in which , the critically ill patient suffers from a haemodynamically significant oxygenation deficit in the tissues without showing obvious changes in the SBP or the HR (Rady *et al.,* 1996). If this deficit in oxygenation occurs as a consequence of inappropriate blood volume it is known as occult hypovolaemia.

1.2.2 Invasive methods

The invasive techniques to estimate volaemia in dogs are based on estimations of the preload or on estimations of the CO (Marshall *et al.*, 2016).

1.2.2.1 Central venous pressure

The central venous pressure (CVP) has been studied as a surrogate of the preload. It measures the pressure in the vena cava, as a representation of the venous return. The volume of blood going back to the right atrium would be equivalent to the volume of blood going into the right ventricle and consequently into the left ventricle (preload) and the systemic circulation (stroke volume), assuming there are no intra or extracardiac shunts or any valve insufficiencies. A central catheter is placed in the vena cava, through the jugular vein, with its tip as close as possible to the right atrium. This central catheter is then connected to a fluid manometer (a water column) or an electric monitor (Oakley *et al.*, 1997). The maintenance of this central catheter can be rather challenging in veterinary patients, with a reported percentage of complications of 51% (Reminga *et al.*, 2018). Intuitively, a high CVP should indicate hypervolaemia or poor cardiac function, while a low CVP should indicate hypovolaemia. However, multiple factors, such as the vascular tone or the intracavitary pressure in the thorax or the abdomen, can affect the CVP, irrespective of the blood volume. As a result, it has been found that CVP correlates poorly with blood volume status and fluid responsiveness in humans (Marik and Cavallazzi, 2013). Fluid responsiveness is a concept widely used in human medicine. It can be described as the ability of a patient to improve his circulatory performance in response to the administration of fluid therapy. Although a study on the correlation of CVP with fluid responsiveness has not been performed in veterinary medicine it is suspected the correlation is also poor (Marshall *et al*., 2016, Drozdzynska *et al.*, 2018).

1.2.2.2 The Fick method

The Fick method was designed to measure the CO. It assumes that all the oxygen inhaled by the lungs is entirely transferred to the arterial blood, and the oxygen remaining in the venous blood is again entirely transferred to the lungs and exhaled. In this scenario, if the oxygen provided to the patient and exhaled by it is known, the measurement of the arterio-venous oxygen content difference will allow calculation of the blood flow, and thus the CO

(Shore *et al.*, 1945). For the oxygen inhaled and exhaled by the dog to be known, it needs to be intubated and placed on a mechanical ventilator. The Fick method is not commonly performed in veterinary patients for several reasons: it is costly, the results are not immediately available, as they require laboratory analyses, and the patient must be anaesthetised, intubated, and ventilated. In addition to the high technical demands, in a haemodynamically unstable patient there is shunting of fluid to compensate for volume changes between the compartments, and the diffusion of oxygen in the lungs can be affected by the actual disease process or by the compensatory mechanisms, making it impossible for the assumptions needed for the technique (complete transfer of oxygen between the blood and the lungs) to be matched. The Fick method is, therefore, more suited for research purposes than for the clinical assessment of critically ill dogs (Marshall *et al.*, 2016).

1.2.2.3 Thermodilution and lithium dilution

The measurement of the dilution of an indicator in the blood stream is another tool to estimate the CO in dogs. The indicator can be a dye or lithium which will change in concentration when they are diluted in the blood stream, or cold saline, which will change in temperature as it dilutes in the blood volume. There are two modalities of the method, depending on how the samples are collected.

In the transpulmonary modality of thermodilution, a known amount of the indicator is injected in one site, frequently the jugular vein, and a blood sample is collected downstream from the original injection site, usually in the femoral artery. The concentration of the indicator in this second location in relation with the initial amount injected will allow to calculate the blood flow, and thus the CO.

In the pulmonary artery modality of thermodilution, a catheter that can sense temperature changes is inserted in the pulmonary artery. A known volume of saline at a known temperature is injected in another site, usually the jugular vein through a central catheter. The temperature change induced in the blood flowing in the pulmonary artery measured by the catheter allows to calculate the dilution that occurred and thus the CO. The two modalities of thermodilution have shown accurate estimation of CO in dogs with fluid overload (Itami *et al.*, 2016). Lithium dilution and transpulmonary thermodilution agreed with pulmonary artery thermodilution in hypo, hyper, and normal haemodynamic states in dogs (Morgaz *et al.*, 2014).

Thermodilution is considered the gold-standard to measure the CO in dogs. However, there could be some complications with the insertion and maintenance of the central catheters (Reminga *et al.*, 2018), and additional risks from the presence of a catheter in the pulmonary artery, like arrhythmias, thrombosis or pulmonary artery rupture (Marshall *et al.*, 2016). Lithium could also be toxic for dogs of small size. Despite the accuracy of these techniques, the necessity of advanced training and equipment make them impractical, and out of reach for most veterinary premises.

1.2.2.4 Pulse contour and pulse pressure analysis

Pulse contour analysis estimates the CO based on calculating the area under the curve of an arterial pulse tracing obtained from an arterial catheter connected to a specific machine containing the required software. A dilution method (lithium or thermodilution) is used to calibrate it, but once it has been calibrated, it provides constant measurements without further injections of indicator. It has shown good agreement with the dilution techniques in anaesthetised dogs (Morgaz *et al.*, 2014), but the arterial catheter needs to be inserted in the femoral artery to provide accurate readings (Shih *et al.*, 2011). The relative changes in stroke volume (SV) calculated by pulse contour analysis showed good ability to predict fluid responsiveness in an experimental model of haemorrhagic shock in mechanically ventilated dogs (Berkenstadt *et al.*, 2005). Other studies, however, showed poor concordance of the pulse contour analysis with pulmonary artery thermodilution (Kutter *et al.*, 2016) and lithium dilution (Cooper and Muir, 2007), and poor ability for the technique to show trends in healthy anaesthetised dogs and anaesthetised dogs subjected to haemorrhagic shock respectively. The presence of arrhythmias or reduced vascular tone affects the quality of the arterial tracing, reducing the accuracy of the technique in clinical patients.

Pulse pressure analysis follows a similar principle to contour analysis with the only difference that it is not calibrated using a dilution method and, thus, it is much less invasive. However, it still requires an arterial catheter to be placed. When tested against thermodilution in dogs, it showed a clear overestimation of the CO and was judged unsuitable for this species (Valverde *et al.*, 2011,Bektas *et al.*, 2012). One study showed that the Vigileo/Flotrac^{TM} monitor, which is based on pulse pressure analysis, may have potential in tracing changes in the SV according to changes in the blood volume (bleeding

or induced volume overload), even though it will not reflect an accurate CO (Taguchi *et al.*, 2011).

Pulse pressure variation (PPV) and systolic pressure variation (SPV) are calculated values obtained from the invasive arterial blood pressure measurements from mechanically ventilated patients. PPV is the result of dividing the difference between the maximum and minimum pulse pressure during one cycle of mechanical ventilation divided by the mean of these values. SPV is the difference between the maximum and minimum arterial SBP during one cycle of mechanical ventilation.

These methods predicted fluid responsiveness in experimental models of mechanically ventilated dogs subjected to haemorrhagic shock (Berkenstadt *et al.* 2005,Westphal *et al.*, 2007, Endo *et al.*, 2017), and in dogs subjected to abdominal surgery (Drozdzynska *et al.*, 2018). PPV showed better performance than SPV. Neither PPV or SPV showed good correlation with the CVP, building on the evidence that CVP holds poor relationship with volume status or fluid responsiveness. The SPV was judged a sensitive indicator of hypovolaemia in an animal model with mechanically ventilated dogs subjected to graded haemorrhage, and it is considered a validated method to estimate cardiac preload in mechanically ventilated dogs (Perel *et al.*, 1987).

Due to the ability of the SPV to estimate cardiac preload, it was tested as a predictor of fluid responsiveness in mechanically ventilated dogs (Rabozzi and Franci, 2014, Sano *et al.*, 2018,Sasaki *et al.*, 2018). The dogs in the study by Rabozzi and colleagues in 2014 were administered a mini-fluid challenge, consisting of the administration of 3ml/kg of a crystalloid, once the patient was under anaesthesia and mechanically ventilated. A change in SPV greater than 4.5% was predictive of a positive fluid response, with haemodynamic improvement after the bolus. Haemodynamic improvement was defined as a 10% increase in SBP, or a 10% decrease in heart rate. The investigators concluded that the SPV can be considered a dynamic index of cardiac preload to guide fluid therapy in anaesthetised, mechanically ventilated dogs.

Sano and colleagues in 2018 used a similar methodology, but the fluid challenge consisted of a 10ml/kg bolus of a colloid, and the animals were considered fluid responders if the SV

estimated from the pulmonary artery flow measured by echocardiography increased by 15% or more. The increased in SV is considered a more accurate surrogate of the haemodynamic performance than the SBP (Cecconi *et al.*, 2014).

Sasaki and colleagues in 2018 also reported good performance of the SPV and PPV to predict fluid responsiveness, defining this as an increase in SV>10%. However, they identified differences on the measurements according to the modality of mechanical ventilation. If the ventilation modality influenced the SPV and PPV, it is very likely, that the changes due to spontaneous breathing will also affect these measurements.

Despite their potential in mechanically ventilated dogs, SPV and PPV would be impractical to measure in most critically ill dogs, which are breathing spontaneously, and may be at risk of complications if they underwent general anaesthesia. In addition to that, the technical requirements to perform these measurements, such as a mechanical ventilator, an invasive arterial BP monitor, and the skill to place an arterial catheter, are seldom available in many veterinary practices.

1.2.2.5 Invasive systolic arterial blood pressure

Systolic arterial blood pressure (SBP) measured by arterial catheterization has failed to demonstrate much value in the assessment of volaemia in dogs in experimental models of haemorrhagic shock (Berkenstadt *et al.* 2005, Westphal *et al.*, 2007) and in dogs undergoing abdominal surgery (Drozdzynska *et al.*, 2018). All three of these studies showed that the changes in blood volume did not correlate with similar changes in the SBP. The explanation for the lack of correlation between blood volume and SBP is in the activation of the compensatory mechanisms during hypovolaemia: RAAS activation, ADH release and increased sympathetic activity. All the compensatory mechanisms working together are very effective in maintaining the SBP irrespective of the blood volume. Thus, the SBP would be mostly related to changes in vascular tone, rather than the blood volume, and thus it is not recommended to assess volaemia.

The fact that SBP correlates poorly with blood volume may put into question the findings of some studies of fluid responsiveness in dogs (Rabozzi and Franci, 2014), that used a

10% increase in SBP as a marker of haemodynamic improvement in anaesthetised and mechanically ventilated patients, in which, the changes in intrathoracic pressure and vascular tone may play a bigger role than the blood volume. In human medicine, the SBP, heart rate and CVP are considered not accurate in predicting fluid responsiveness (Cecconi *et al.*, 2014, Boyd *et al.*, 2016, Monnet *et al.*, 2016a).

1.2.3 Non-invasive methods

The non-invasive techniques measure surrogates of blood volume without accessing the intravascular space to estimate the actual volume status.

A non-invasive variation of the Fick method has been used in dogs to estimate the CO. The dog needs to be anaesthetised, intubated, mechanically ventilated, and connected to a rebreathing system with carbon dioxide sensors. The differences between the concentrations of inhaled and exhaled carbon dioxide are used to calculate the CO. It has shown good correlation with lithium dilution in healthy anesthetized dogs (Gunkel *et al.*, 2004). However, the necessity for general anaesthesia and mechanical ventilation makes it impractical for clinically ill dogs.

Bioimpedance is based on measuring changes in the electrical resistance of a circuit to the movement of electricity through it. In this case the circuit is the thorax, which will oppose varying degrees of resistance depending in the amount of fluid that is present in it, mostly the blood flow in the aorta, allowing the calculation of the CO. A clinical study (Yamashita *et al.*, 2007) showed poor correlation between bioimpedance and thermodilution to estimate CO in dogs. Another study, however, showed good correlation with thermodilution in an experimental model of cardiac surgery in dogs (Sasaki *et al.*, 2017). The main limitation for this technique is the necessity of specific and costly machinery, which is very sensitive to movement and noise (Marshall *et al.*, 2016) and thus impractical for veterinary clinical patients.

Bioreactance is based on similar concepts to bioimpedance. An electrical current is applied to the thorax, but in this case, instead of the resistance, it measures the change in the frequency of the current as it travels through the circuit. It showed very good correlation with thermodilution in dogs to estimate the CO in an experimental model (Heerdt *et al.*,

2011). However, it has not been tested in clinical veterinary patients yet, and the same limitations of bioimpedance regarding availability and cost of the machinery, apply.

Plethysmography variability index is a value obtained from the waveform of a pulse oximeter reading. It is calculated through a mathematical algorithm and it is thought to be influenced by preload. An experimental study in mechanically ventilated dogs showed that increases in this value over a certain threshold can accurately predict fluid responsiveness (Endo *et al.*, 2017). Although the technique is not invasive, it requires the application of a pulse oximetry probe to the tongue or lip, which can be rather challenging in conscious animals. Other locations for the placement of the probe of the pulse oximeter, such as the tail, ear, and toe, have been tested and they seem to provide acceptable readings (Huss *et al.*, 1995). However, this requires for the contact between the probe and the vascular bed to be optimal and for the animal to be immobile, as this was tested in anaesthetised Beagles. Thus, obtaining a reading of enough accuracy and quality to be measured in a conscious dog, may still be difficult.

Additional non-invasive methods based on ultrasound have been used scarcely in veterinary medicine and very extensively in human medicine. They will be discussed in further detail in a separate section.

1.2.4 Laboratory measurements

Some laboratory parameters can be useful in assessing the relative changes in the plasma volume in dogs suffering from non-blood loss hypovolaemia, even though they do not provide any estimation of the CO.

1.2.4.1 The packed cell volume

The packed cell volume (PCV) measures the percentage of the blood volume represented by all the blood cells. Erythrocytes represent the majority of the blood cell population, therefore, the PCV can be used interchangeably with the haematocrit, which measures the percentage of the blood volume represented by the erythrocytes. When the blood cell volume remains constant, but the plasma volume decreases, most commonly from dehydration, the value of the PCV increases in a phenomenon called relative erythrocytosis (Ettinger *et al.*, 2016). An in-line haematocrit monitor, that provided serial measurements,

has been proven useful to estimate the changes in blood volume induced by the administration of resuscitative fluid therapy in an experimental model in healthy anaesthetised dogs (Silverstein *et al.*, 2005). Thus, PCV relative changes can be useful to monitor the response to treatment in non-blood loss hypovolaemic states in dogs.

1.2.4.2 The total protein

The total protein (TP) measures the amount of protein dissolved per unit of plasma. In cases of dehydration, it follows a similar pattern to the PCV. A reduction of the plasma volume will increase the TP (Ettinger *et al.*, 2016). The administration of fluid therapy and restoration of the plasma volume should induce a decrease in its value. However, there are many different types of proteins with different functions and diffusion capabilities included in the TP, which can prevent accurate repeated measurements (McGrotty and Knottenbelt, 2002). They can move through the fluid compartments, but they do so with different ability depending on their molecular weight and three-dimensional structure. Protein losses through the urine or the intestinal secretions can be exacerbated during pathological states and again will be conditioned by their molecular characteristics. Acute phase proteins would be produced and released into the intravascular space in systemic inflammatory processes. All these factors make the relative changes in TP during hypovolaemia and after fluid therapy treatment very challenging to monitor. Therefore, this parameter is mostly used in conjunction with the PCV to assess changes in the plasma volume when both values trend in the same direction.

The albumin concentration in plasma (ALB), as an individual type of protein, can be more useful in monitoring relative changes in blood volume. A contraction of the plasma volume will increase the ALB, and the administration of fluid therapy should reduce its concentration (Davis *et al.*, 2013). A simultaneous decrease in all these three parameters (PCV, TP and ALB) was documented when the plasma volume of healthy anaesthetised dogs was expanded through the administration of different protocols of fluid therapy (Muir *et al.*, 2011).

1.2.4.3 Lactate and acid-base balance

The diagnosis of shock can be supported by laboratory parameters, such as the lactate concentration in plasma (LAC) and the acid-base analysis. As discussed earlier, shock is defined as a disruption of the oxygen supply to the tissues. Lactate is mostly a by-product of the anaerobic glycolysis. Therefore, its production would increase as the oxygen supply to the tissues decreases in what is called type A hyperlactataemia (Sharkey and Wellman, 2013). Conditions that induce a disruption of the carbohydrate metabolism can also elevate LAC, irrespectively of an appropriate oxygen supply, which is called type B hyperlactataemia. Hypovolaemic shock induces type A hyperlactataemia as the inappropriate intravascular volume reduces peripheral perfusion and thus, the oxygen supply to the tissues (Pang and Boysen, 2007). The compensatory mechanisms triggered during hypovolaemia (RAAS activation, ADH secretion, and increased sympathetic activity) can maintain normal LAC until the early decompensated stage of shock. Thus, hyperlactataemia is a late sign of hypoperfusion. The increase in LAC seems to have a close to linear relationship to the level of tissue hypoxia in dogs. (Gillespie *et al.*, 2017). An effective treatment should reduce LAC, and a failure to do so has been linked with poor prognosis in dogs presented as emergencies, irrespectively of the underlying condition (Stevenson *et al.*, 2007). However, the decrease in LAC does not follow the same linear relationship and it can take several hours for the treatment to have an impact on LAC. Therefore, its prognostic value is currently considered superior to its diagnostic value (Rosenstein *et al.*, 2018).

Acid-base analysis in dogs with hypovolaemic shock can present a wide range of alterations. The most common is the presence of metabolic acidosis, mostly linked to increases in LAC and low oxygen content. However, an experimental model of haemorrhagic shock in dogs proved that there are many other anions involved in the development of strong acidosis (Bruegger *et al.*, 2007). However, if the hypovolaemic shock has been induced by excessive gastric losses, this can have an alkalinizing effect, thus masking the acidosis. Acid-base abnormalities can be sustained, even after apparently successful volume resuscitation (Young *et al.*, 2014) and are therefore challenging to use to monitor volume status or response to treatment in hypovolaemic states.

1.3 The role of ultrasound for the assessment of volaemia and the presence of shock in human medicine

Ultrasound technology has been available for human medicine for decades, and it is widely used for multiple purposes in the emergency room and critical care unit. This fact triggered an interest towards the use of these devices to monitor the haemodynamic status of the critically ill human patient. Several ultrasonographic measurements, protocols and techniques have been developed in recent years (Bernier-Jean *et al.*, 2017) and are currently replacing more invasive techniques, such as thermodilution, as a first-line diagnostic and monitoring tool for most critical patients (Cecconi *et al.*, 2014).

1.3.1 Measurements of the inferior vena cava for the assessment of volaemia in humans

This method rapidly gained popularity due to its technical simplicity (Feissel *et al.*, 2004, Stawicki *et al.*, 2009, Martin *et al.*, 2013). It is based on the concept that the diameter of the major veins and the fluctuations of this diameter can reflect the volume status and predict the fluid responsiveness of the patient. Several different vessels have been investigated, but the inferior vena cava (IVC) has been the most widely studied, followed by the subclavian vein and the superior vena cava (Kent *et al.*, 2013).

The maximal diameter of the IVC, and the changes in the diameter of the vessel during the respiratory cycle, or more recently, the absolute maximal and minimal diameters, irrespectively of the phase of the respiratory cycle, have been used to calculate the collapsibility index (CI), according to the formula: IVC-CI = $[(IVC_{max} - IVC_{min})/ IVC_{max}] \times 100$. The IVC diameters and IVC-CI have shown good correlation with the pressure in the right atrium measured through a flotation catheter, and with the invasive CVP (Kircher *et al.*, 1990, Bodson and Vieillard-Baron, 2012). However, it has been stated recently that the CVP correlates poorly with the fluid responsiveness (Marik and Cavallazzi, 2013). Thus, the IVC measurements may also correlate poorly, which is what has been found in the more recent reviews and meta-analysis (Long *et al.*, 2017, Orso *et al.*, 2020).

The performance of the caval measurements was substantially different in different populations of patients. The studies that looked at surgical and emergency patients showed better performance than those that looked at patients in intensive care units (Dipti *et al.*,

2012). This may be a consequence of the different underlying conditions and the influence of interventions such as mechanical ventilation or invasion of the body cavities. For instance, the variation of the IVC measured in mechanically ventilated people in septic shock, was proposed as a useful tool to guide fluid therapy (Feissel *et al.*, 2004). The predictive ability for fluid responsiveness of the respiratory variation of the IVC diameter was greater in mechanically ventilated patients, than in those breathing spontaneously (Long *et al.*, 2017). This meta-analysis by Long and colleagues in 2017 suggests that although it can be useful in certain subpopulations, it is still not applicable to the whole human population.

It is mentioned in the systematic reviews (Zhang *et al.,* 2014, Long *et al*., 2017, Orso *et al.*, 2020) , that there are noticeable differences between studies on how the measurements of the IVC were performed, employing different acoustic windows, and different positions of the patient, all of which can greatly influence the measurements (Mookadam *et al.*, 2011). How fluid responsiveness can be measured objectively has been, and continues to be, a source of controversy (Ansari *et al.*, 2016). Thus, it is difficult to find studies that are completely comparable, as they can use different definitions to establish fluid responsiveness. Most of them will use thermodilution, CVP, or echocardiography to define a positive fluid response, and see how the IVC measurements behaved. Again, the lack of a standardized gold-standard to compare against, makes the different studies difficult to compare.

In summary, even though there are promising indications for the measurements of the IVC in the assessment of haemodynamic status and response to fluid therapy in several subpopulations of human patients, the technique needs more standardization and further study, as the current data suggests that it is not useful to predict fluid responsiveness (Millington, 2019, Orso *et al.*, 2020).

1.3.2 Echocardiography for the assessment of volaemia in humans

Echocardiography is currently the standard of care recommended by the consensus on circulatory shock and haemodynamic monitoring of the European Society of Intensive Care Medicine (Cecconi *et al.*, 2014). Echocardiography has been referred to as, textual citation from McLean 2016: "the most single useful tool in the diagnosis and management of shock". This technique has also been sought as being accurate in the prediction of fluid responsiveness (Boyd *et al.*, 2016) and it is currently recommended for this purpose by the guidelines of the British Society of Echocardiography (Miller *et al.*, 2016) and the American Society of Echocardiography (Porter *et al.*, 2015). When compared with thermodilution, the current gold-standard, it showed good performance in estimating CO (Zhang *et al.*, 2019). For all these reasons, it can be safely said, that echocardiography is currently the most useful tool for the assessment of volaemia in human patients, with a performance almost as good as the invasive methods. Thus, invasive techniques are being replaced by echocardiography as the standard of care (Cecconi *et al.*, 2014).

Several different modalities of echocardiography can be used for the estimation of CO: transthoracic echocardiography, transoesophageal echocardiography (TOE), and the ultrasonic CO monitor (USCOM). The latter is a simplified ultrasonographic device that has a Doppler beam that can be placed over the chest of the patient and provides a Doppler spectrum of the aortic flow. Echocardiography estimates the CO appraising the SV by either of these two methods:

- Multiplying the velocity-time integral (VTI) of the Doppler spectrum of the aortic or pulmonary forward flow times the cross-sectional area of the aorta. This method has already been validated for research purposes (Moulinier *et al.*, 1991).
- Calculating the difference between the left ventricular end diastolic and end systolic volumes.

The USCOM monitor and TOE showed better performance than the transthoracic modality in estimating the CO, possibly due to better alignment with the aortic flow, but all three modalities correlated well with thermodilution (Zhang *et al.*, 2019). When trying to predict fluid responsiveness or estimate blood volume, not only the measurement of the variations of the SV is useful (Marik *et al.*, 2009), but also other data from the echocardiographic exam.

The observation of an obliterated lumen of the left ventricle (LV) is indicative of hypovolaemia (Tavernier *et al.*, 1998, Feissel *et al.*, 2001). Changes in the size of the LV measured by TOE, reflected changes in preload, although this was not a good predictor of fluid responsiveness (Cannesson *et al.*, 2006, Marik *et al.*, 2011). Hypovolaemia can induce diastolic dysfunction (Chew, 2012), which will increase the risk of volume overload when administering fluid therapy. This could be prevented by using echocardiography to assess diastolic function before the administration of fluids. A dilated right ventricle can be a sign of pulmonary thromboembolism (obstructive shock) or volume overload (Mercat *et al.*, 1999). An increase in size of the right ventricle without an increase in the left ventricular SV is an objective end-point for fluid therapy (Miller *et al.*, 2016).

When there are still doubts about the fluid responsiveness of the patient, there are some manoeuvres that can increase the accuracy of echocardiography in predicting fluid responsiveness: a fluid challenge, a passive leg raising test, and a study of the variation in SV during mechanical ventilation.

A fluid challenge is the rapid administration of intravenous fluid. A change in the VTI of the aortic flow of 10% after administering 100ml of a colloid over one minute predicts positive fluid responsiveness with a sensitivity of 95% and a specificity of 78% (Muller *et al.*, 2011). The passive leg raising test consists in measuring the CO or the SV after raising the legs of the patient, in order to increase the venous return to the right atrium. It is applicable to mechanically ventilated and spontaneously breathing patients. During this test, an increase in the CO or SV by 12% or more is predictive of fluid responsiveness, with slightly better performance for the changes in SV (Monnet *et al.*, 2016b). When patients are under mechanical ventilation, a SV variation of 10% over the respiratory cycle under a ventilated tidal volume of 8 to 10ml/kg is highly predictive of positive fluid responsiveness (Marik *et al.*, 2009). Smaller tidal volumes have not been investigated, so patients are transiently moved to this range of volumes to make this assessment and then readjusted to their optimal values after the procedure.

In addition to its usefulness in estimating fluid responsiveness, and monitoring the patient response to fluid therapy, echocardiography is employed in human patients to establish the aetiology of shock. The most used classification of circulatory shock used in human medicine (Vincent and De Backer, 2013) comprises of four main categories: cardiogenic,

hypovolaemic, distributive and obstructive. Each category has specific echocardiographic characteristics, as can be seen in Table 1.3.

Table 1-3. Classification of circulatory shock in humans. Adapted from Vincent and De Backer 2013.

Shock category	**Echocardiographic features**	**Examples**
Hypovolaemic	Small chambers and normal to high contractility	Haemorrhage Severe dehydration
Cardiogenic	Large ventricles and poor contractility	Myocardial failure Arrhythmias
Distributive	Normal chambers and normal or low contractility	Sepsis Anaphylaxis
Obstructive	In tamponade: pericardial effusion, small chambers and dilated IVC. In pulmonary thromboembolism: dilated right ventricle and small LV	Pericardial effusion Thromboembolism

It is recommended by the consensus on circulatory shock and haemodynamic monitoring of the European Society of Intensive Care Medicine (Cecconi *et al.*, 2014) to use echocardiography under a suspicion of shock to:
(1) better characterize the haemodynamic disorders
(2) select the best therapeutic option (IV fluids, positive inotropes, or ultrafiltration)
(3) assess the response of the haemodynamic disorders to therapy.

This consensus (Cecconi *et al*. 2014) defined fluid responsiveness in human patients in shock as an increase of 10 to 15% in the stroke volume (SV), estimated from the velocity-time integral (VTI) measured by Doppler echocardiography of the aortic forward flow. This same consensus discourages the use of invasive techniques such as thermodilution or pulmonary artery catheterization as a first line and reserves them for patients not responding to the therapy chosen based on echocardiography. It also states that useful information can be obtained from echocardiography in around two minutes by non-cardiologists after minimal training (Beraud *et al.*, 2013). Even just a visual assessment,

without measurements, can help clinicians in estimating cardiac systolic function (McGowan and Cleland, 2003).

Summarizing, echocardiography is currently the standard of care in human medicine to identify the underlying mechanism of shock, to predict fluid responsiveness and to monitor response to treatment after the administration of fluid therapy.

1.4 Volume status assessment using ultrasound in dogs.

Ultrasound techniques have been growing in popularity in recent years as the equipment has become available in many veterinary practices. Ultrasound examination to assess volaemia in veterinary medicine has tried to adapt human techniques to veterinary patients. In this scenario, the same modalities of ultrasound have been employed: vessel diameter assessment, TOE, other Doppler devices, including USCOM, and transthoracic echocardiography.

1.4.1 Measurement of the caudal vena cava for the assessment of volaemia in dogs

The measurements of great vessels in dogs for the assessment of volaemia have focussed almost exclusively on the caudal vena cava (CVC). The ultrasonographic technique to explore this vessel was first described along with other abdominal vessels (Finn-Bodner and Hudson, 1998) and it was stated that in the author´s opinion its size was dependent on patient´s size, moment of the respiratory and cardiac cycles, and hydration. It was later included as part of a triage ultrasonographic protocol in the emergency room (Boysen and Lisciandro, 2013), which proposed that a subjective dilation of the CVC and the hepatic veins could be suggestive of right sided heart failure. Thus, a full echocardiography may follow, to confirm this finding.

The CVC to aorta ratio (CVC/Ao), was designed to obtain a value independent of body size in children (Kosiak *et al.*, 2008), and was adopted in dogs for the same reasons, as there is a big heterogenicity in the body weight of the canine population (Meneghini *et al.*, 2016). Meneghini and colleagues in 2016 proved a good correlation between the changes in the CVC/Ao ratio and the SPV after a fluid bolus administered in one minute in mechanically ventilated anaesthetised dogs. These dogs were subjects of skin wound repairs, therefore there was no invasion of their body cavities. This is an important detail, because a laparotomy can change the intraabdominal pressure, which may influence the diameter of compressible vessels such as the CVC. They also obtained the images of both vessels (CVC and Ao) simultaneously, in a transverse right-lateral intercostal view, after suspending temporarily the mechanical ventilation. The CVC/Ao ratio increased in these animals after the administration of the fluid bolus.

The CVC/Ao ratio was validated for the assessment of volemia in dogs subject to a blood donation (Cambournac *et al.*, 2018), demonstrating lower values for the CVC/Ao after the extraction of 9.8 ± 2.2 mL/kg of blood. This was due to a marked reduction in the diameter of the CVC, while the Ao remained almost unchanged. This was expected, because as discussed earlier, the compensatory mechanisms are very effective in maintaining the SBP, which will intuitively keep the diameter of the Ao stable, whereas hypovolaemia will decrease the pressure in the right atrium, and therefore in the CVC, reducing its diameter. They obtained the diameter of both vessels simultaneously in a transverse view. A low intra and interobserver variability was reported, although only two different operators took the measurements. Thus, they proposed this ratio may be of use in detecting reductions in blood volume.

Two recent studies, have challenged the theory that the CVC/Ao ratio can detect reductions in blood volume in dogs (Marshall *et al.*, 2018, Herreria-Bustillo *et al.*, 2019). Marshall and colleagues in 2018 stated that when measuring CVC/Ao ratio in nine greyhounds subjected to an 8% blood loss, the change in these measurements, even though of statistical significance, was of such a small magnitude that it may not predict changes in real clinical cases. They employed transverse and sagittal views to measure the CVC. Although reductions of the diameter were seen in both views, this was of a bigger magnitude in the sagittal views. The CI was also included in this study, and proved to be smaller after blood loss, but again this change was of such a small magnitude (≈0.02) that it may not be identifiable in clinical cases. The inclusion of only nine animals and of only one breed, with breed-specific peculiarities in regards to their blood volume (Courtice, 1943) may imply these results were not applicable to the general canine population.

Herreria-Bustillo and colleagues in 2019 failed to demonstrate any difference in the CVC/Ao ratio before and after a blood donation of one unit of whole blood in greyhounds. They used an M-mode to do the measurements, instead of the 2D mode described in other studies, making the results difficult to compare. The small population size included in this study (eight greyhounds) and the presence of only one breed, with higher blood volume than other breeds (Courtice, 1943) may have resulted in a non-representative sample of the general canine population.

In a different study, the induction of volume depletion in healthy Beagle dogs by the administration of 1mg/kg of IV furosemide produced significant reductions in the CVC/Ao ratio (Kwak *et al.*, 2018). Transverse and longitudinal views were obtained. The authors described that it was subjectively easier to identify the maximum diameter of the CVC in the transverse view. Only one operator acquired all the images, preventing any assessment about the inter-operator variability of the technique. Several different ratios were obtained, employing the area of the CVC, its maximal height and width in the transverse view, and its maximal height in the longitudinal view. The values for all those ratios were smaller after volume depletion and also different between animals with or without clinical signs of dehydration. Thus, this study concluded that it could potentially be a method for the estimation of volaemia. The same limitation about studying only one breed of dogs applies.

A very recent study (Rabozzi *et al.*, 2020), which was published after the study design and data acquisition for the current study were completed, measured the CVC/Ao ratio and the CVC CI in an heterogenous population of hospitalized conscious dogs. They employed transverse images of both vessels at the same time at the porta hepatis, and a short and long axis of the CVC at the hepatic vein inlet, with the dog in left lateral recumbency. They defined positive fluid responsiveness as an increase of 15% in the VTI of the aortic forward flow after a fluid challenge consisting of an IV bolus of 4ml/kg of Hartmann´s solution administered over one minute. They obtained the Doppler spectrum of the aortic flow at the subxiphoid view in right lateral recumbency. They were able to predict fluid responsiveness with a sensitivity of 100% and a specificity of 75% with a threshold value of 0.83 for the CVC/Ao in this population of conscious dogs. However, the authors emphasised that an echocardiography alongside the measurement of the CVC/Ao is advisable to overcome some of the limitations of this ratio such as the changes induced in it by right sided heart failure, pleural effusion, cardiac tamponade, pulmonary thromboembolism, or pneumothorax. The CVC CI measured in this study did not show significant differences after the fluid challenge, and the authors discouraged the use of this index to assess volaemia or predict fluid responsiveness in conscious dogs.

The most recent study (Donati *et al.*, 2020) performed in spontaneously breathing dogs was a retrospective study that reviewed the patients that received a fluid challenge consisting of the administration of a 30ml/kg bolus of a crystalloid IV, and had complete medical records of the VTI and CVC CI measurements. They defined fluid responsiveness

as an increase in the VTI of the aortic forward flow of 15% or greater. They acquired the aortic flow from the left apical window in left lateral recumbency. The CVC was assessed in long axis from the right transhepatic window, and the measurements were taken in M-mode. They reported a 100% sensitivity and 83.3% specificity to predict fluid responsiveness for the CVC CI when using a threshold of 27%. Interestingly, they did not find significant differences between the maximal diameter of the CVC adjusted to body weight between responders and non-responders, suggesting that this measurement is of no value to predict fluid responsiveness.

Reference values for the CVC diameter, area and CVC/Ao ratio in healthy dogs from different views have been established recently (Darnis *et al.*, 2018). Not all acoustic windows performed equally. The subxiphoid view, which is the standard for human patients (Mookadam *et al.*, 2011), showed a lower inter-rater agreement than the hepatic and paralumbar views. The measurements obtained in 2D mode showed better agreement that those in M-mode. The hepatic view, although showing good agreement, interrogated a vessel that was elliptical in shape. Therefore, the use of the area of the vessel was more accurate than the diameter alone. The reference ranges provided by this study were very wide, but it was hypothesized by the authors that during hypovolaemia or hypervolaemia the measurements of the CVC will be comprised in a narrower range.

Summarizing, the ease and rapidity of the procedure, and the shortness of the learning curve needed to obtain repeatable measurements of the CVC (Darnis *et al.*, 2019), makes it attractive for intensive care clinicians in veterinary medicine. However, its clinical applicability is still under debate and, to date, very few studies have been performed in clinical veterinary patients. In addition to that, most studies were performed in only one breed of dog, limiting even more the applicability of their results to the general canine population. As it happens in human medicine, there is a lack of standardization of the technique to acquire and measure the images. In addition to that, the variables used to define positive fluid responsiveness also differ between studies, making them difficult to compare.

1.4.2 Echocardiography for the assessment of volaemia in dogs

As in humans, several techniques and devices have been trialled in dogs using ultrasound to assess the volume status.

1.4.2.1 Transoesophageal echocardiography and other ultrasound-based techniques

The CO estimation obtained from TOE has shown good to excellent agreement when tested against thermodilution in mechanically ventilated anaesthetised dogs (Yamashita *et al.*, 2007, Mantovani *et al.*, 2017). When tested simultaneously with other non-invasive techniques, such as bioimpedance and partial CO_2 rebreathing (a modified Fick method), TOE showed the best correlation with thermodilution (Yamashita *et al.*, 2007). However, this study included only six dogs, and they were all of the same breed (Beagle). The CO of these six Beagle dogs was manipulated through the administration of a dobutamine infusion, so they were not clinical cases. The CO was estimated from the Doppler tracing of the aorta (CO = HR x (VTI x Ao area), which can be very well aligned with TOE, making this acoustic window more suited in dogs than in humans, where the alignment with the flow is more difficult due to the anatomy of the thorax.

Similar findings were reported in another study with anaesthetised and mechanically ventilated dogs of different breeds (Mantovani *et al.*, 2017). Using the same methodology as Yamashita and colleagues 2007, but a different acoustic window, Mantovani and colleagues 2017 observed an excellent agreement between TOE and thermodilution during normotension. Good, but lower agreement was seen during isoflurane-induced hypotension. Most patients with hypovolaemia can maintain an adequate arterial SBP, due to the activation of the compensatory mechanisms, thus, this should not be a limitation in such cases.

A simplified oesophageal ultrasonographic Doppler device was tested in healthy anaesthetised dogs to estimate CO. This oesophageal Doppler device showed poor correlation with the thermodilution in healthy anaesthetised dogs (Canfrán *et al.*, 2015). In addition to that, as any other transoesophageal technique, it requires general anaesthesia, so it would be impractical for critically ill conscious dogs.

The USCOM device was tested in two experimental models with anaesthetised Beagle dogs. While one of them showed good correlation between the CO measured by USCOM and by a high-precision transit time ultrasonic flow probe placed on the ascending aorta (Critchley *et al.*, 2005), a later study found poor correlation with thermodilution (Scansen *et al.*, 2009). There is a technical limitation when using this device in dogs, as the software included in the machine estimates the aortic cross section based on the height of a human patient. When used in dogs, the aorta should be measured by echocardiography and this value inserted in the software to generate more accurate readings. It has not been used in clinical veterinary patients yet.

1.4.2.2 Transthoracic echocardiography

Transthoracic echocardiography has been used to study changes in blood volume in dogs. The influence of the blood volume on transthoracic echocardiographic variables has been already reported.

One study showed that experimentally induced volume depletion or dehydration in healthy dogs of different breeds produced noticeable reductions in the cardiac chamber sizes measured by transthoracic echocardiography (Fine *et al.*, 2010). Volume depletion in this study was induced by the administration of furosemide, while dehydration was induced by water deprivation for eight hours. These changes were of different magnitude and in different variables in the dogs treated with furosemide than in the dogs deprived of water. While the left atrial dimension, left ventricular end-systolic and end-diastolic volumes, SV, M-mode LV diastolic diameter, E wave velocity, and aortic peak pressure gradient, were significantly reduced in the furosemide treated group, only the left ventricular diastolic volume and the left atrial size were reduced in the dehydrated dogs. This was interpreted by the authors as a proof that volume depletion will cause more marked changes in the cardiac chambers than pure dehydration due to the compensation through an increased osmolarity of the plasma. However, the weight loss in the furosemide group was greater, making the two groups not completely comparable. Thus, it is possible than the furosemide group showed more marked changes as a consequence of having a more pronounced hypovolaemia.

This would suggest echocardiography could be useful in diagnosing and grading hypovolaemia in dogs. Similarly designed studies proved the same reductions in chamber sizes and alterations in systolic and diastolic variables in other veterinary patients, such as horses (Underwood *et al.*, 2011) and cats (Campbell and Kittleson, 2007, Sugimoto *et al.*, 2019).

Two case series described the development of reversible left ventricular outflow tract obstruction as a consequence of hypovolemia in dogs. This feature disappeared in all these patients once the blood volume was restored (Aoki *et al.*, 2015, Hammes *et al.*, 2016). Two of the three dogs presented in these case series were diagnosed with hypovolaemic shock due to a splenic rupture, while another one was severely dehydrated. The authors of both case series hypothesized that the reduction in the lumen of the left ventricle was a sign of reduced preload, and that it narrowed the left ventricular outflow tract, causing systolic anterior motion of the mitral valve. Additionally, the secretion of catecholamines and the compensatory mechanisms triggered in the course of shock would increase cardiac contractility, thus inducing the obstruction.

The reversibility of these changes and the absence of signs of cardiac disease after volume replacement in these three patients suggested that transthoracic echocardiography can identify hypovolaemia in dogs euvolaemia, irrespectively of the aetiology.

The purpose of all the forementioned studies in this section was to prove the influence of blood volume status on the echocardiographic measurements, in order to avoid erroneous echocardiographic diagnosis of heart diseases such as hypertrophic cardiomyopathy or diastolic dysfunction in animals that may be volume depleted or dehydrated. However, they did not assess the performance of transthoracic echocardiography to estimate blood volume.

The estimation of the CO using transthoracic echocardiography in dogs, has been very scarcely reported, and has shown conflicting results so far.

Two studies demonstrated good to excellent agreement between the CO measurements provided by a flowmeter inserted in the aorta and the CO estimated from echocardiography.

One of these studies, was performed in open-chested, mechanically ventilated, anaesthetised dogs (Steingart *et al.*, 1980)with the ultrasound transducer held next to the aorta through a thoracotomy. This is not in the usual position from outside the chest, limiting the applicability of these results to transthoracic echocardiography.

The second study(Uemura *et al.*, 2013) was performed in ten close-chested, anaesthetised and mechanically ventilated dogs, using transthoracic echocardiography and the peripheral arterial pressure profile to estimate the CO. The authors proved a good agreement in the CO measurements between the flowmeter and echocardiography, and also a good trending capacity of the latter during several haemodynamic conditions, such as hypotension, fluid overload and cardiac pacing. They used a modified approach to calculate the SV, measuring the maximal velocity from the aortic flow profile, and the ejection time from the peripheral pulse wave. In that way, they eliminated the need for an operator to manually trace the aortic profile. The main limitation of this technique is that it needed calibration, using the flowmeter, before readings could be accurately taken. The authors proposed the thermodilution method to do the first calibration and continue the monitoring with this minimally invasive protocol. The need of an initial calibration makes the technique impractical for the general canine population.

Two studies have tested transthoracic echocardiography against thermodilution. One of these studies (Day *et al.*, 2007) reported a lack of agreement between these two techniques, whereas the other one (Lopes *et al.*, 2010) reported a clinically acceptable correlation between them. The dogs included in both studies were anaesthetised healthy individuals. Additionally, Day and colleagues subjected the dogs to a blood loss of 45ml/kg. Although the CO estimation from echocardiography was judged suboptimal, Day and colleagues observed qualitative changes in the heart chambers and the great vessels as the blood loss progressed. They hypothesized that the changes in geometry in the ventricles induced by these changes may have affected their echocardiographic flow profiles.

Both studies reported a better performance for the pulmonary flow than for the aortic flow. For the acquisition of the aortic flow profile, both studies positioned the animals in the left lateral recumbency and used an apical five-chamber view which is not consider the optimal view to perform aortic forward flow interrogation (Abbott and Maclean, 2003).As the

Doppler interrogation technique is angle dependant this may have influenced the results, resulting in a poorer agreement,. Day, and colleagues also hypothesized, that the changes in the aortic diameter after haemorrhage may have affected the calculated CO. In disagreement with this hypothesis, most studies have identified no changes in the aortic diameter in relation to changes in blood volume (Meneghini *et al.*, 2016, Bucci *et al.*, 2017, Rabozzi *et al.*, 2020)

Day and colleagues made a special emphasis on the statistical methods they employed in this study, applying the Bland-Altman plots, instead of correlation, which has been traditionally used to assess the agreement between techniques. In this description of the statistical analysis, they suggested that the lack of agreement between the techniques did not imply transthoracic echocardiography was unsuited to detect clinical changes associated with blood volume reduction, but only that echocardiography was not suited to accurately measure CO during haemorrhage, which may be of importance in a research environment, but may not be so relevant in a clinical setting. Correlation was the statistical method employed by Lopes and colleagues, which may also explain the divergent results, as they concluded that the pulmonary artery forward flow provided a clinically acceptable non-invasive means of estimating CO. If the pulmonic flow was clinically acceptable it is possible than the aortic flow would also be so, if the echocardiographic technique is optimised.

No studies have been performed to assess the usefulness of transthoracic echocardiography for the assessment of volaemia or fluid responsiveness of conscious, spontaneously breathing, clinically ill dogs. As invasive and advanced techniques for the assessment of volaemia and fluid responsiveness are seldom available in veterinary facilities, this project will evaluate a variety of cardiac measurements that can potentially be useful for this purpose in dogs.

The aim of the current study would be to assess if transthoracic echocardiography can be useful in the estimation of volaemia in conscious, spontaneously breathing, critically ill dogs. A second aim would be to develop a simple and brief ultrasonographic protocol that can be performed by non-cardiologist veterinarians, aiding in the diagnosis of hypovolaemia, and fluid responsiveness.

2. Materials and methods

2.1 Equipment

The following equipment was employed to perform this research project:

- Packed cell volume (PCV) measurement kit (Thomas scientific, USA)
- Lactate meter: Stat strip lactate Xpress meter (Woodley veterinary diagnostics, UK)
- Ultrasound machine: Mindray M5 (Shenzhen Mindray biomedical electronics, China)
- Refractometer (RS components, Czech Republic)
- Doppler flow detector CAT+ Doppler blood pressure kit (Thames medical, UK)
- Multiparameter anaesthetic monitor with oscillometric SBP meter: GE B40 (General Electric, USA)

2.2 Animals, diagnostic tests, and ultrasonographic protocol

This was a prospective cohort study. The author obtained ethical approval from the ethics committee of the University of Glasgow. Owners provided informed consent for echocardiography and other clinically indicated procedures, at the time of presentation. Owners also permitted use of their pet's anonymised clinical data for research purposes.

2.2.1 Animals

The population of the study were dogs presented to one single private veterinary hospital in the UK. They were divided into two different cohorts of patients:

1. Control cohort: included dogs referred for echocardiography to a cardiology referral in a private veterinary hospital in the UK. They were included in the control group if the data from the history and physical examination suggested they presented a normal volume status (euvolaemic), there were no clinical signs of any systemic disease, and the echocardiography showed no signs of substantial cardiac disease. The absence of clinical signs or alterations in the physical exam deemed laboratory tests unnecessary. The SBP was measured by non-invasive methods, either Doppler or oscillometry in either the forelimb or the tail. Only the SBP was recorded after averaging the results of three to five measurements. Substantial cardiac disease was defined as any cardiac

disease that caused remodelling of the heart chambers. The patients included were dogs with innocent murmurs or dogs affected by mild degenerative mitral valve disease whose cardiac chambers were considered subjectively normal and whose cardiac chamber measurements were within the limits of the stage B1 according to the American College Veterinary Internal Medicine (ACVIM) Consensus (Keene et al. 2019). This means their left ventricle and left atrium were not significantly enlarged and did not meet the criteria described in this consensus for the definition of cardiomegaly. All dogs were scanned conscious and spontaneously breathing. Dogs were placed in right and then left lateral recumbency and underwent a full standard echocardiography. The ultrasonographic exploration of the CVC was performed in right lateral recumbency from the subxiphoid window. Electrocardiography leads were attached to the limbs to produce a simultaneous electrocardiogram tracing. One single clinician assessed all the animals, performed the ultrasonographic exam, acquired all the images, and made all the measurements.

2. Study cohort: comprised dogs presented to the out of hours service of a private hospital in the UK. They were assessed by the same clinician that examined the dogs from the control cohort. They were included in the study cohort if the history and physical exam suggested they had hypovolaemia or hypovolaemic shock. There were no exclusions based on the underlying disease. The suspicion of hypovolaemia was originally based on the history and the physical examination after assessing mucous membranes colour (MM), capillary refill time (CRT), heart rate (HR), and pulse quality. The dogs of the study cohort were suspected to have mild or moderate hypovolaemia based on the history if the owner had reported obvious fluid losses such as bleeding, profuse vomiting, or persistent diarrhoea. The dogs were suspected of having hypovolaemic shock if the owner reported altered mentation or collapse. In the data from the physical examination: pulse quality and MM were classified in four different categories, which are detailed in the Table 2.1. Capillary refill time was considered prolonged if it exceeded 2 seconds. HR was considered elevated if it was over 160 bpm. Dogs in the study cohort were suspected of being in hypovolaemic shock if there was altered mentation or collapse, in addition to white or pale MM, markedly elevated HR (over 180 bpm), markedly prolonged CRT (≥3seconds) or weak pulses. It was not required that all the variables matched this description, but instead a general clinical picture was drawn from the history and the physical examination in order to advise the owners to

perform further testing. If that was deemed necessary, owners were asked to give permission to perform a SBP measurement, a blood test and the ultrasonographic protocol that was the subject of this research project.

Table 2-1. Categories of MM and pulse quality

Variable	**Description**	**Category**
MM	white	3
	pale	2
	pink	1
	congested	0
pulse quality	not palpable	3
	weak	2
	fair	1
	strong	0

2.2.2 Diagnostic tests

After signed consent was obtained from the owners, the SBP was measured by non-invasive indirect methods, either Doppler or oscillometric. The same method, and the same site were always used for the same dog for the subsequent measurements. The measurement sites were the forelimb or the tale. Only the SBP was recorded after averaging the results of three to five measurements. Hypotension was diagnosed if the SBP≤90mmHg, and hypertension if the SBP≥160mmHg.

Blood tests were performed after obtaining a blood sample from venepuncture of the jugular vein. If the clinical condition of the animal was judged very unstable the blood sample was drawn from the venous catheter that was inserted in the cephalic vein in order to provide fluid therapy. Laboratory tests comprised: PCV, TP in plasma, and LAC in whole blood. The PCV was expressed as percentage and measured manually using a PCV kit after spinning a microhaematocrit tube for 5 minutes in a microhaematocrit centrifuge at 12,000 revolutions per minute. The TP in plasma was measured manually in a refractometer, previously calibrated with water, after depositing one drop of the centrifuged plasma from the microhaematocrit tube. The units read from the refractometer (g/100ml) were converted into grams per litre (g/L). The LAC was measured in whole blood by a bedside strip-based meter from the blood remaining in the needle from the

blood extraction and expressed in millimole per litre (mmol/L). Lack of appropriate tissue perfusion was suspected if LAC≥2.5 mmol/L. Additional blood tests were performed in most patients, such as full biochemistry and haematology, but these were not included in the study.

2.2.3 Ultrasonographic protocol

Once the non-invasive SBP measurement was performed and the blood sample drawn, dogs in the study cohort underwent an ultrasonographic exploration, comprising of a simplified echocardiographic protocol and the CVC exploration. The ultrasonographic exploration was performed in conscious dogs, breathing spontaneously in right lateral recumbency. Whenever possible, electrocardiogram leads were attached, but this was based on patient´s needs, as some animals required immediate intervention. The time consumed by the examination was recorded. Two echocardiographic windows were used to perform all the ultrasonographic views and acquire all the cine loops that were used to obtain all the measurements. The following windows and views were recorded:

- Subxiphoid window (Figure 2-1). It was performed to obtain the longitudinal subxiphoid view of the CVC (Figure 2-2) and the subxiphoid view of the heart for Doppler examination of the aortic forward flow (Figure 2-3).
- Right parasternal window (Figure 2-4). It was performed to obtain the following echocardiographic views: long-axis four chambers view optimised for the left atrium (L4chA) (Figure 2-5), long-axis four chambers view optimised for the left ventricle (L4chV) (Figure 2-6), long-axis five chambers view (L5ch) (Figure 2-7), and short-axis view at the level of the papillary muscles (Spm) (Figure 2-8).

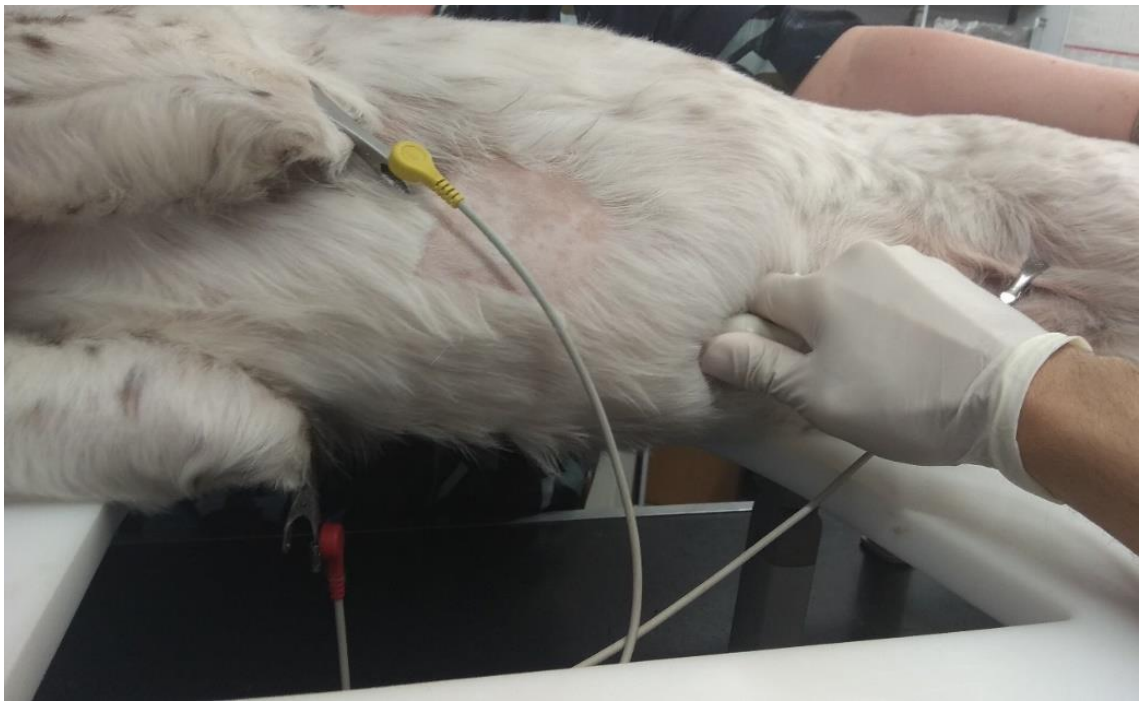

Figure 2- 1. Control dog held in right lateral recumbency with electrocardiogram leads attached. Operator scanning using the subxiphoid window.

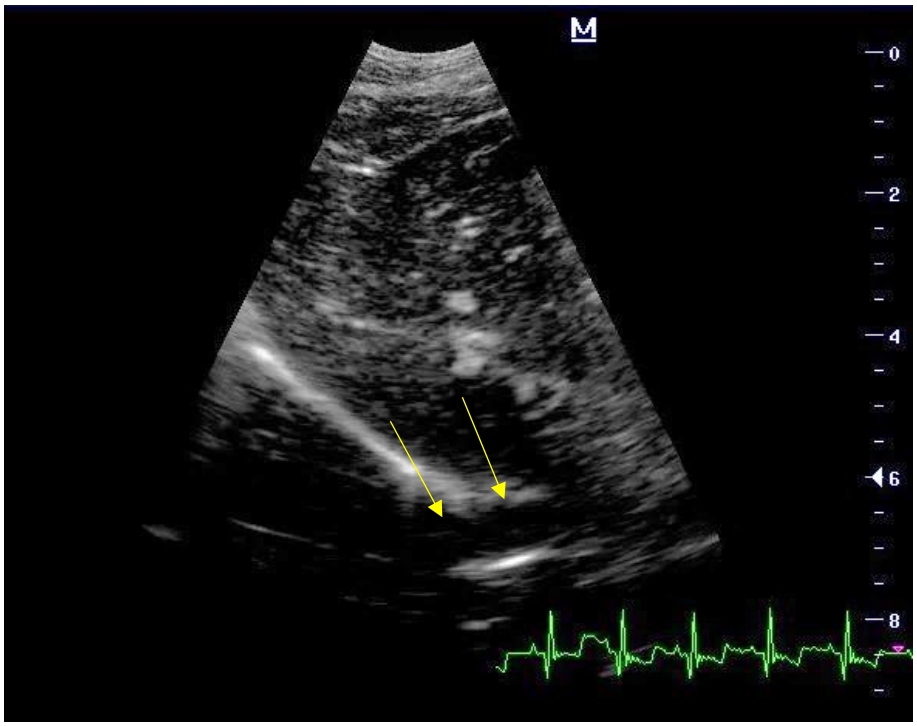

Figure 2-2. Longitudinal subxiphoid view of the caudal vena cava (arrows) from the subxiphoid window.

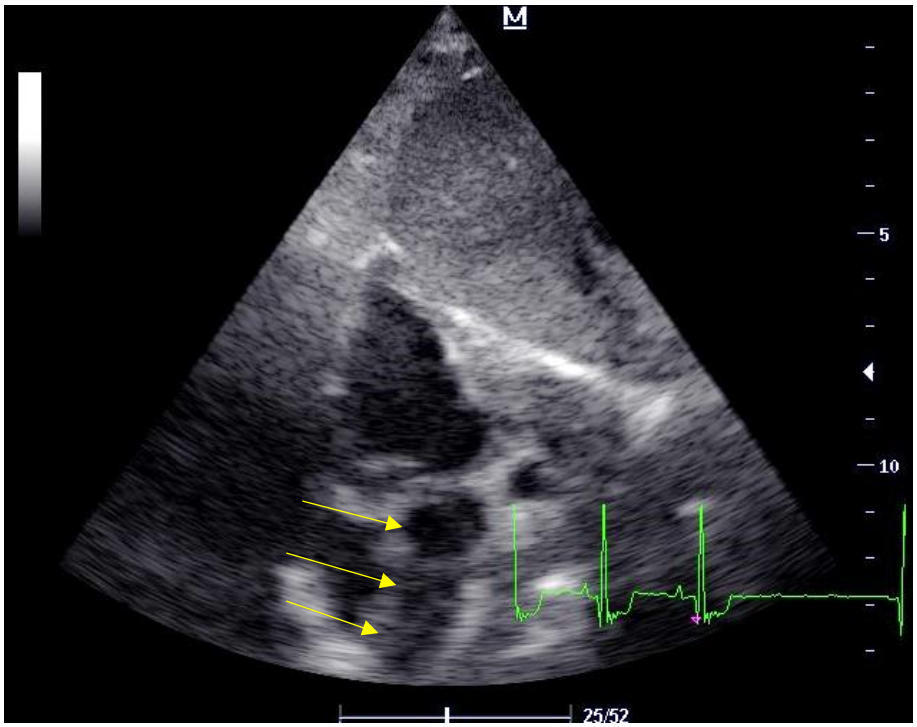

Figure2-3.Subxiphoid view of the aorta (arrows) from the subxiphoid window.

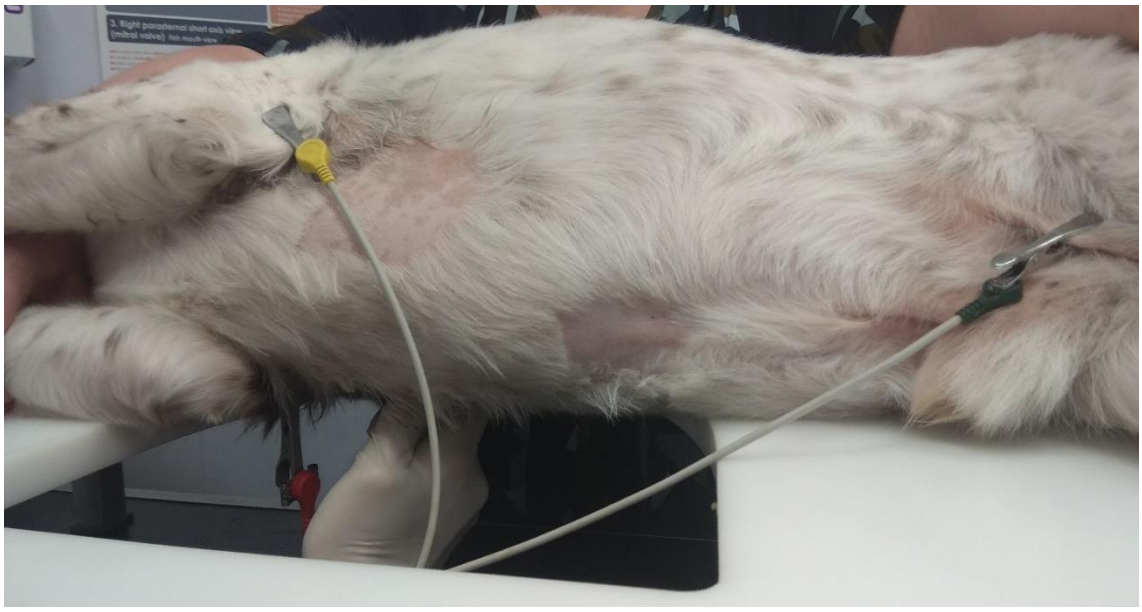

Figure 2-4. Control dog held in right lateral recumbency with electrocardiogram leads attached. Operator scanning using the right parasternal window.

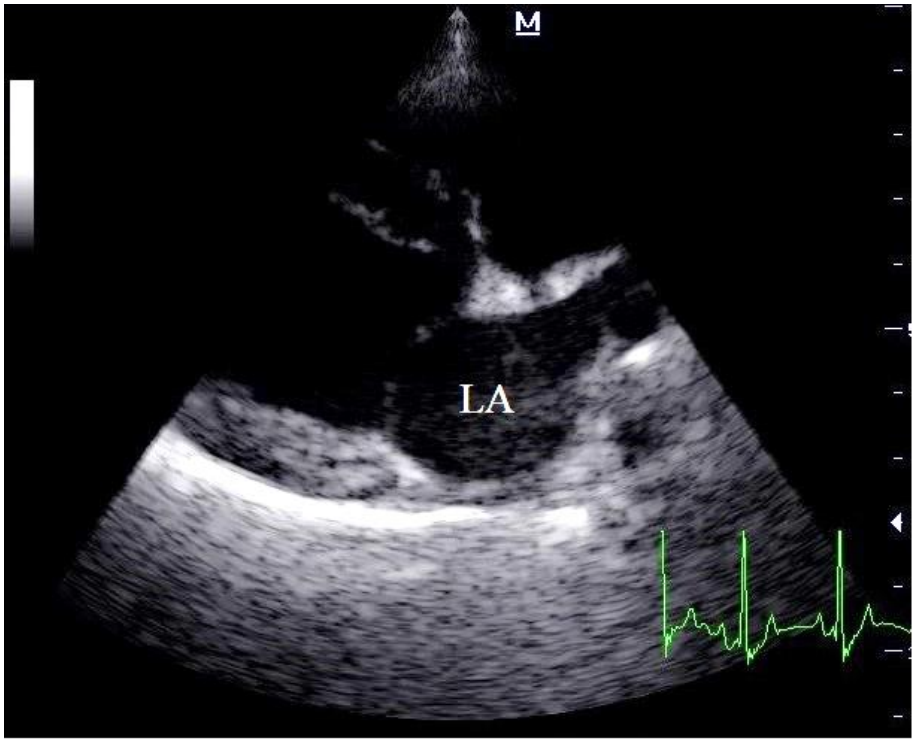

Figure 2-5. Long-axis 4 chamber view optimised for the left atrium (LA) from the right parasternal window.

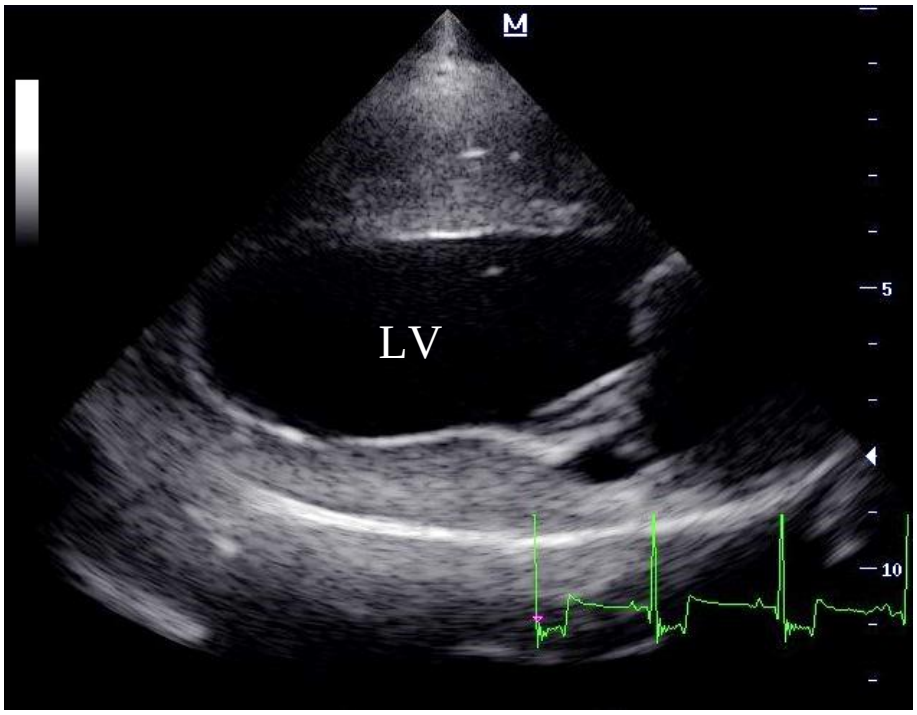

Figure 2-6. Long-axis 4 chamber view optimised for the left ventricle (LV) from the right parasternal window.

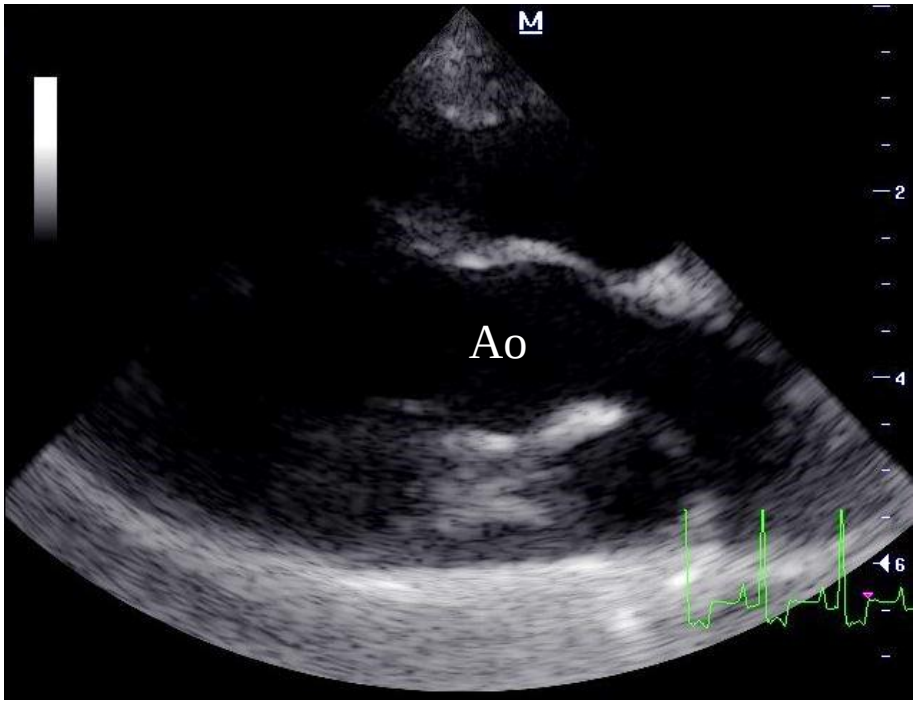

Figure 2-7. Long-axis 5 chamber view from the right parasternal window showing the aorta (Ao).

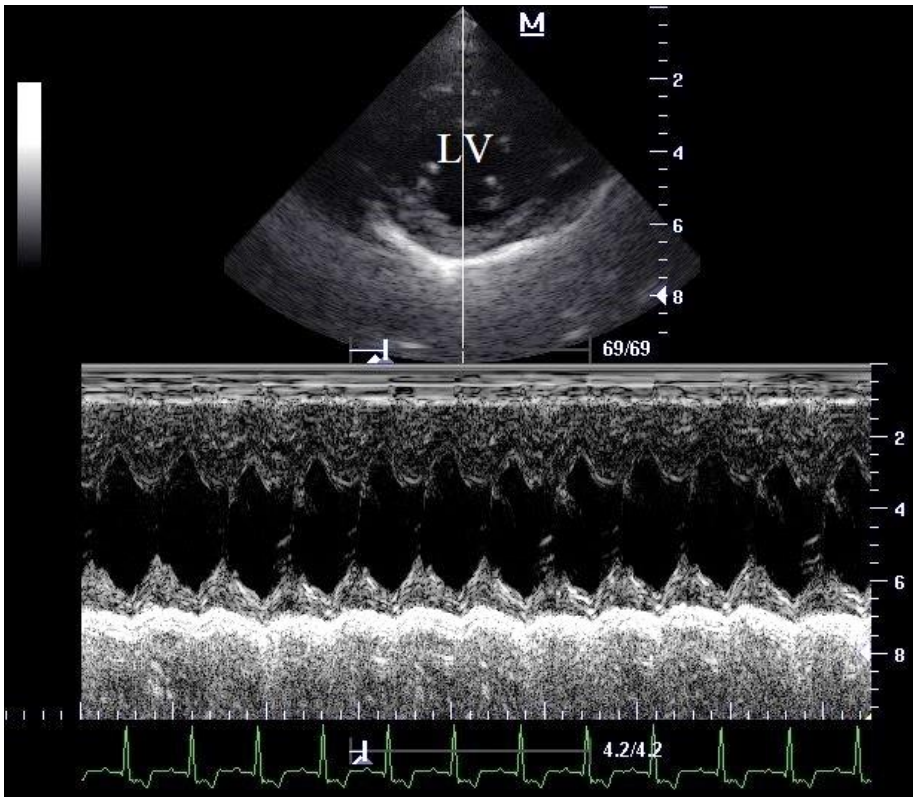

Figure 2- 8. Short axis view of the left ventricle (LV) at the level of the papillary muscles from the right parasternal window.

Once the cine loops were acquired, the measurements were performed off-line at a later time. Three measurements were made in three different cardiac cycles and averaged to obtain the value included in the study. One single operator, the same as for the control cohort, acquired all the images and performed all the measurements. They comprised:

- Left atrial major longitudinal diameter to aortic diameter ratio (LA_{major}/Ao). (Strohm *et al.*, 2018) The major longitudinal diameter of the left atrium (LA_{major}) was measured in the L4chA view, tracing a line from the interatrial septum to the lateral atrial wall parallel to the mitral annulus in the last frame before the opening of the mitral valve as described by Strohm and colleagues in 2018 (Figure 2-9). The aortic diameter was measured in the L5ch view tracing a line between the hinges of the aortic valve in systole (Figure 2-10).

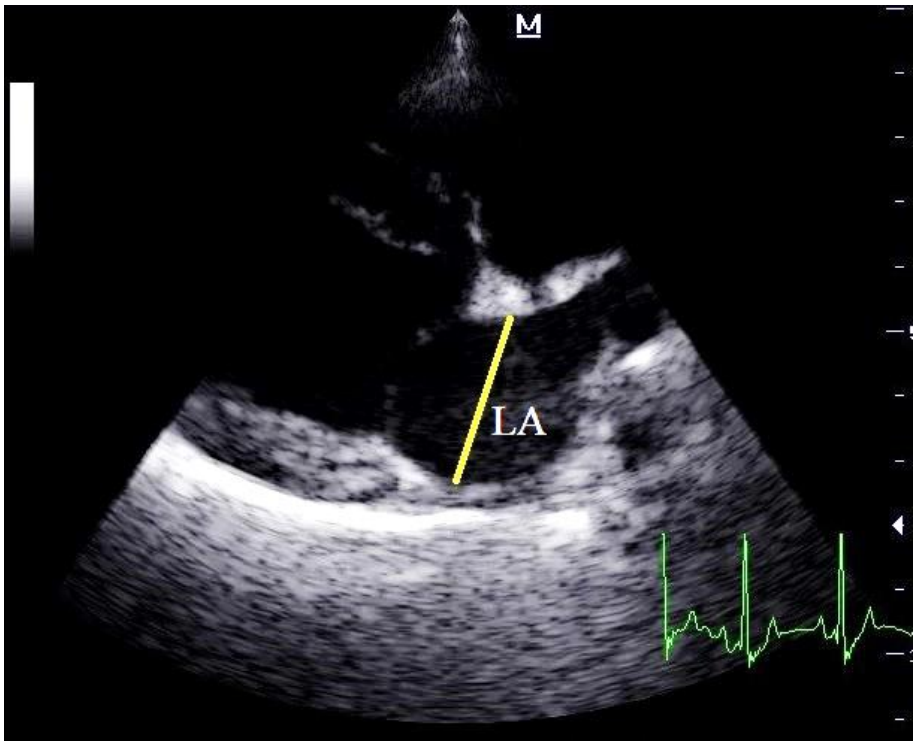

Figure 2- 9. Right parasternal 4 chamber view. The yellow line represents the measuring plane for the major longitudinal diameter of the left atrium (LA).

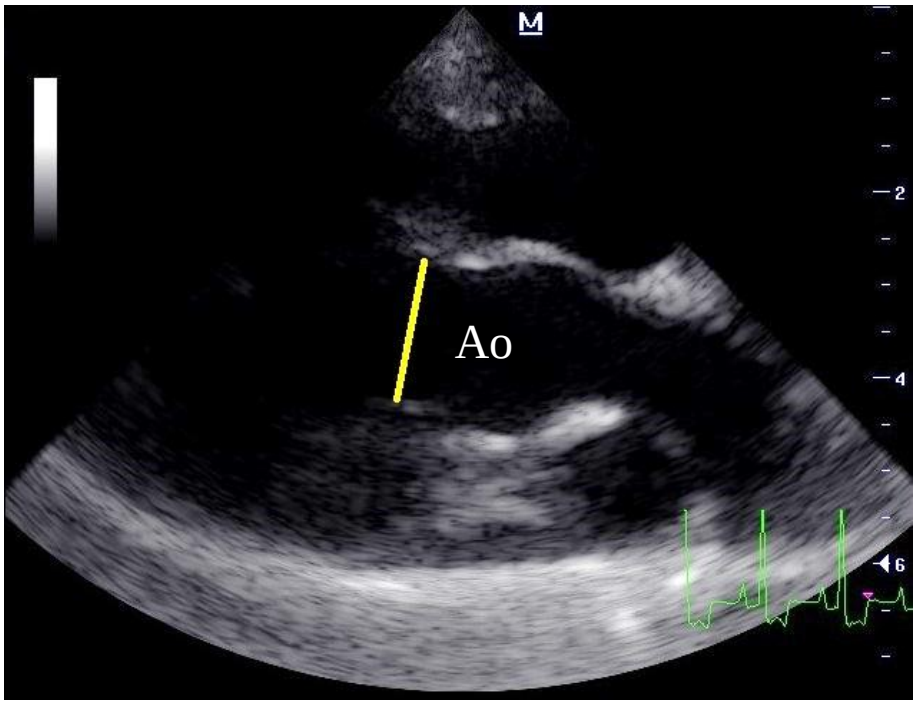

Figure 2-10. Right parasternal 5 chambers view. The yellow line represents the measuring plane for the aortic diameter between the hinges of the aortic valve. (Ao) aorta.

- Left ventricular end diastolic volume index (EDVI). The diastolic area of the LV in the L4chV view was traced following the endocardial border excluding the papillary muscles, if present, in the first frame after the mitral closure (Figure 2-11). The Simpson´s method of discs was applied using the cardiac software of the ultrasound machine to obtain the end diastolic volume, which was divided by the body surface area to obtain the EDVI (Wess *et al.*, 2010). In addition to the measurements of the left ventricular volume, the L4chV view was used to subjectively assess the presence of left ventricular obliteration.

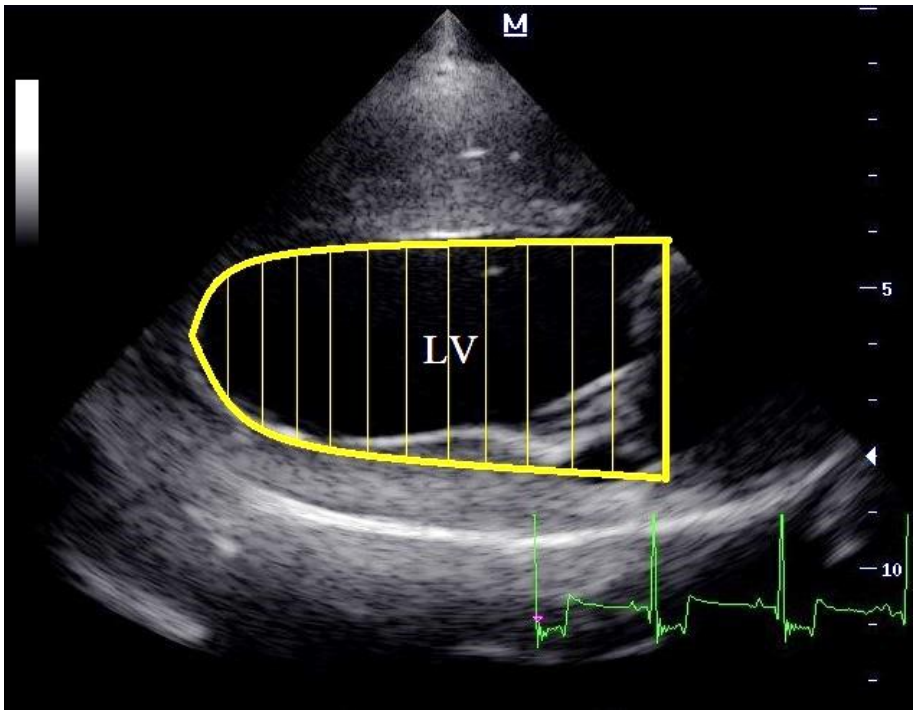

Figure 2-11. Right parasternal 4 chambers view optimised for the left ventricle (LV). The yellow area represents the measuring technique employed, following the endocardial border to trace the end diastolic area. The Simpson´s method of discs was used to calculate the end diastolic volume.

- Left ventricular internal diameter in diastole normalized for body weight by allometric scaling (LVIDdN). The left ventricular internal diameter during diastole (LVIDd) was measured following the "leading edge to leading edge" methodology at the onset of the Q wave of the simultaneous electrocardiogram tracing, in an M-mode tracing guided by the 2D mode in the Spm view (Figure 2-12) (Bonagura, 1983). The LVIDd was normalized to body weight following allometric scaling (Cornell *et al.*, 2004) according to the formula:

$$LVIDdN = LVIDd/BW^{0.294}$$

Where BW is body weight.

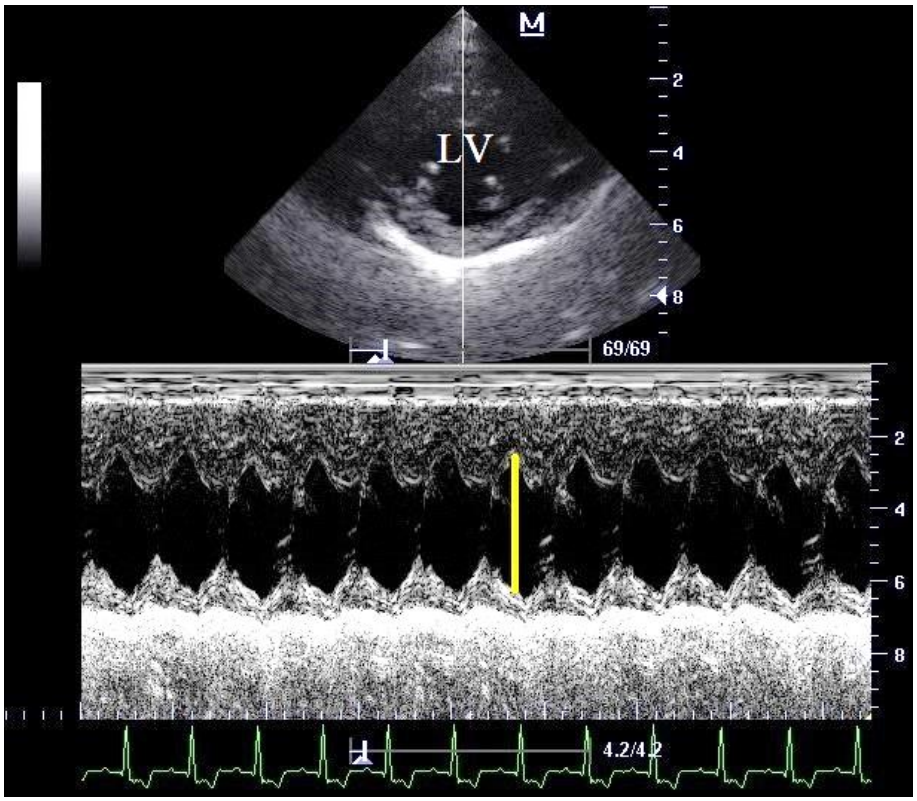

Figure 2-12. M-mode tracing obtained at the right parasternal short axis view of the left ventricle (LV) at the level of the papillary muscles. The yellow line represents the measuring plane for the left ventricular internal diameter in diastole.

- Stroke volume (SV) in cubic centimetres (cm^3). This was calculated according to the following formula (Phillips *et al.*, 2017):

$$SV = VTI \times Ao\ area$$

Where *VTI* is the velocity-time integral, in cm, of the Doppler tracing of the aortic forward flow from the subxiphoid view (Figure 2-13), calculated by the cardiac software of the ultrasound machine, and *Ao area* is the area of the aortic annulus in cm^2, calculated from the aortic diameter previously measured in the L5ch view (Figure 2-10) according to the formula.

$$Ao\ area = \pi r^2$$

Where "r" is the result of diving the aortic diameter measured in the L5ch view by two to obtain the radius of the aorta.

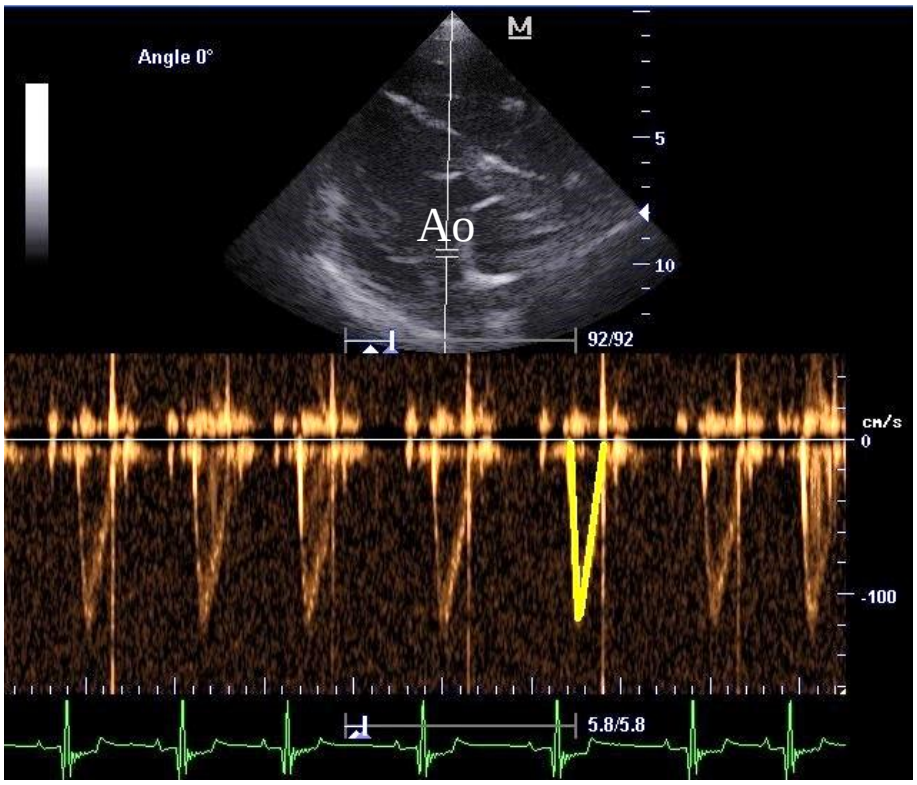

Figure 2-13. Doppler spectrum of the aortic forward flow obtained from the subxiphoid view. The yellow lines draw the velocity-time integral of the aortic flow. (Ao) aorta

- CVC maximum diameter (CVC_{max}). A longitudinal plane of the CVC was obtained from the subxiphoid view in right lateral recumbency (Figure 2-2), and a cine loop was recorded when the vessel appeared subjectively at its biggest diameter. The CVC was measured in millimetres (mm), on its maximal diameter, tracing a perpendicular line from the inner edge to the inner edge of the walls of the CVC at its crossing of the diaphragm (Figure 2-14), regardless of the moment in the cardiac or respiratory cycle (Darnis *et al.*, 2018). In addition to the measurements, the longitudinal subxiphoid view of the CVC was used to subjectively assess the presence of dilation or collapse of the CVC.

- CVC_{max} to aorta ratio (CVC_{max}/Ao). Where CVC_{max} is the maximum diameter of the CVC previously obtained from the subxiphoid view, and Ao is the aortic diameter previously obtained from the L5ch view. (Cambournac *et al.*, 2018, Kwak *et al.*, 2018).

- CVC collapsibility index (CVC CI). This was calculated following the formula:

$$CVC\ CI = \frac{CVCmax - CVCmin}{CVCmax} x\ 100$$

Where CVC_{min} is the minimum diameter of the CVC obtained from the same longitudinal subxiphoid view as the $CVC_{max,}$ but on its narrowest diameter, irrespectively of the moment of the respiratory cycle (Darnis *et al.*, 2018).

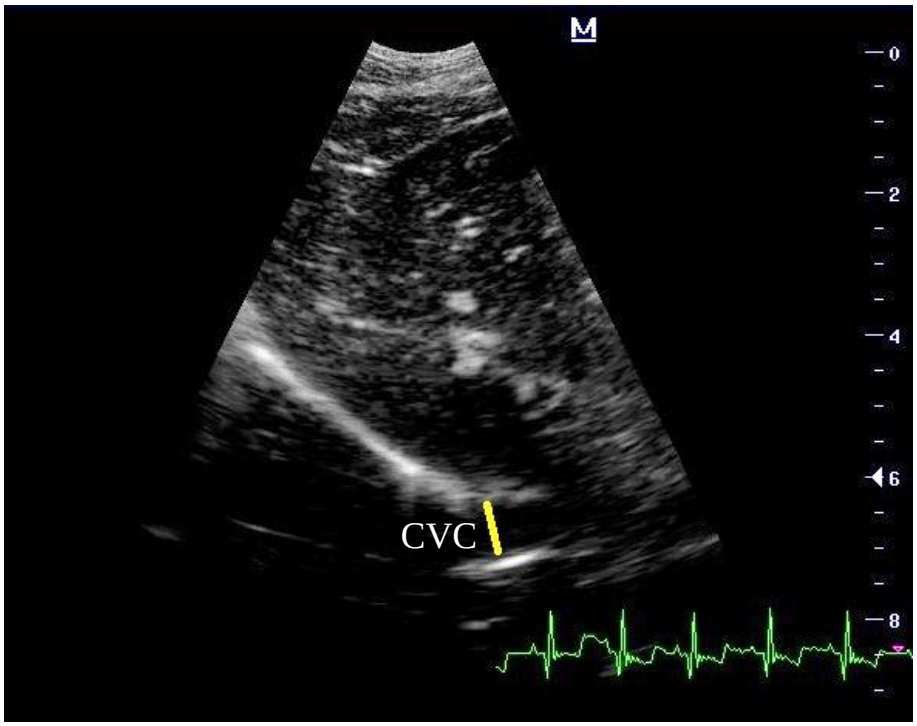

Figure 2-14. Subxiphoid view of the caudal vena cava (CVC). The yellow line represents the measuring plane of the vessel at its crossing of the diaphragm, used to measure its maximal and minimal diameters.

Once the ultrasonographic examination was completed, an intravenous catheter was placed in the cephalic vein and fluid therapy was administered intravenously (IV). The IV fluid therapy was tailored according to the patient´s needs based on the 2013 AAHA/AAFP Fluid therapy Guidelines for dogs and cats (Davis *et al.*, 2013), and was recorded as per fluid type, volume, and time of administration. All the variables of the physical exam, SBP, laboratory tests and ultrasonographic measurements were reassessed after the administration of the chosen protocol of IV fluid therapy. This was a fluid bolus in a few minutes if the animal was considered in shock, or replacement fluid therapy for dehydrated animals given over a period of 24 hours.

Animals were classified as fluid responders if they achieved an increase of 10% or greater in the SV, as described in human medicine in the current consensus on circulatory shock and haemodynamic monitoring of the European Society of Intensive Care Medicine (Cecconi *et al.*, 2014).

2.3 Statistical analyses

The data were analysed using commercially available software Microsoft Excel Analysis data Pack tool, Microsoft Office 365 and Minitab Statistical Software, Minitab LLC, USA.

Most data were not normally distributed, according to the Shapiro-Wilk test, so non-parametric tests were applied. All the descriptive statistics are presented as median and interquartile range 25^{th}-75^{th}, employing this format for all the variables assessed in the study and for all the demographic characteristics of the population. The level of significance was set at $P \leq 0.05$ for all tests.

Logistic regressions were used to compare the demographic characteristics (age, body weight and sex distribution) between cohorts.

The Kruskal Wallis test was used to compare the control versus the study cohort. Binary logistic regressions were used to identify which parameters better discriminate between the control and study cohorts, and a non-parametric ROC curve was employed to identify cut-off values.

The study sample size for the best chance to obtain a significant result, when comparing the medians of the study and control cohorts with a power of 0.8, $\alpha = 0.05$ and $\beta = 0.05$ was nineteen observations. This was based in published data about the variations of the CVC size (Rabozzi *et al.*, 2020) in a similar subpopulation of dogs, where the population and sample variances were expected to be similar.

Data from the study cohort were compared before (pre-treatment) and after treatment (post-treatment) using a Wilcoxon signed-rank test.

Among the study cohort, animals were classified as fluid responders, if they achieved a 10% increase in the SV, or non-fluid responders if they did not reach this 10% increase in the SV. A Mann-Whitney U test was used to compare the measurements pre-treatment and post-treatment of these two sub-groups.

Among the study cohort, animals were classified as being in shock or not in shock. A Mann-Whitney U test was used to compare the measurements pre-treatment and post-treatment of these two sub-groups.

2.4 Subjective assessment

The subjective assessment of the cardiac chambers and CVC diameter were performed at the time of the ultrasonographic examination. This was performed by the same operator that acquired the images and made the measurements.

The subjective appearance of the CVC in the longitudinal subxiphoid view was categorized as dilated, normal, or collapsed. The CVC was described as dilated if the diameter of the vessel seemed subjectively big compared with the diameter of the aorta and there was scarce change on the diameter throughout the cardiac cycle. The CVC was categorized as normal if the diameter changed over the cardiac cycle, with a noticeable difference between maximal and minimal diameters, and the maximal diameter seemed somehow smaller than that of the aorta. The CVC was described as collapsed if the lumen of the vessel was almost completely obliterated at some point during the cardiac cycle and the maximal diameter was clearly smaller than the aortic diameter.

The LV was assessed for the presence of lumen obliteration. The degree of obliteration was categorized as severe, moderate, or not present. Severe obliteration was noted when the free wall of the LV and the interventricular septum touched, or almost touched each other during systole. Moderate obliteration was described if one or both of the walls of the LV were bent towards the lumen of the ventricle during diastole. A subjectively normal lumen for the LV during systole and diastole was classified as the obliteration was not present.

The correlations of these subjective changes with lack of adequate tissue perfusion, defined as a LAC≥2.5mmol/L, fluid responsiveness, and shock were studied observationally, as the small number of dogs prevented statistical analyses.

3. Results

3.1 Statistical results

3.1.1 Control cohort vs study cohort

The control cohort consisted of nineteen dogs. These nineteen dogs had an age of 9 (8-11) years, and a median body weight of 14 (8-22) kilograms (kg). Many breeds were represented: Cocker spaniel, Staffordshire terrier, Labrador (n=3), Shih-Tzu, Yorkshire terrier, Pointer, Cavalier king Charles spaniel, Podenco, Border collie, Tibetan terrier, Chihuahua, Jack Russell, Beagle, and mix-breed (n=4).

The study cohort comprised of eighteen dogs. These eighteen dogs had an age of 9 (4.5-11) years and a body weight of 13 (8-19) kg. Many breeds were represented: Cocker spaniel (n=2), Staffordshire terrier, Great Dane, Border collie, Pekinese, Doberman, Cairn terrier, Lurcher, Cavalier king Charles spaniel (n=2), Dachshund, Shetland sheepdog, Boxer, West highland white terrier, Lhasa Apso, Springer spaniel, and mix-breed.

The demographic characteristics of both cohorts are presented in Table 3-1. There were no significant differences in age, body weight and sex distribution between the cohorts.

Table 3-1. Demographic characteristics of the control and study cohort presented as median (interquartile range). P value obtained by logistic regression.

Variable	**Control cohort (n=19)**	**Study cohort (n=18)**	**P value**
Age (years)	9 (8-11)	9 (4.5-11)	0.440
Body weight (kg)	14 (8-22)	13 (8-19)	0.869
Sex distribution	males: 10 females:9	males:12 females:6	0.512

.

In the control cohort, none of the dogs presented clinical signs at the time of examination. The physical exam was unremarkable, except for the presence of a heart murmur grade III/VI or lower in fourteen out of the nineteen dogs. Five out of the nineteen dogs did not have a murmur. Given the fact that there were no clinical signs nor significant findings in the physical examination they were consider euvolaemic; they did not undergo further

testing other than echocardiography and the non-invasive measurement of the SBP. Their values for the elected physical exam variables and SBP are presented in Table 3-2. Among the study cohort, the most common clinical presentation was the presence of intense gastrointestinal signs (10/18), comprising vomiting, diarrhoea, or both. This was followed by collapse (n=6), prolonged anorexia (n=1) and lethargy (n=1). Their values for the elected physical exam variables and SBP are summarised in Table 3-2.

The HR was higher (p= 0.004), and the CRT was longer (p=0.048) in the study cohort at the time of presentation, than in the control cohort. There were not significant differences between the control cohort and the study cohort at the time of presentation in the SBP.

Table 3-2. Values for continuous variables of the physical examination and the systolic blood pressure in the control cohort and study cohort at the time of presentation. P value obtained by logistic regression.

Variables	**Value control cohort**	**Value Study cohort at presentation**	**P value**
CRT (s)	2 (2-2)	2 (2-3)	0.048
HR (bpm)	116 (104-125)	152 (131-160)	0.004
SBP (mmHg)	140 (130-159)	130 (119-152)	0.133

.

In the categorical variables, MM colour and pulse quality, the control cohort grouped all its dogs in the categories considered normal (strong pulses (0) and pink MM (1)), whereas the study cohort had representation in all categories (Table 3-3).

Table 3-3. Number of dogs included in each category of the categorical variables pulse quality and mucous membranes colour in the control and study cohort.

Variable	**Categories**	**Control cohort**	**Study cohort pre-treatment**
Pulse quality	0	19	6
	1	0	6
	2	0	5
	3	0	1
MM colour	0	0	3
	1	19	9
	2	0	5
	3	0	1

.

In the study cohort at the time of presentation, eleven of the eighteen dogs were suspected of varying degrees of hypovolaemia, with no presence of shock based on the physical exam findings and laboratory variables. Seven of the eighteen dogs were suspected to be in hypovolaemic shock.

The ultrasonographic measurements of the control cohort and the study cohort at the time of presentation are summarised in Table 3-4. The LA_{major}/Ao was smaller (p=0.034) in the study cohort at the time of presentation than in the control cohort. The EDVI (p=0.064), and the LVIDdN (p=0.068), were not significantly different between cohorts, although they were approaching significance. The SV (p=0.448) and the measurements of the CVC were not significantly different between the control and study cohorts.

Table 3-4. Ultrasonographic measurements of the control cohort and study cohort at the time of presentation. P value obtained by Kruskal-Wallis.

Variables	**Control cohort**	**Study cohort at presentation**	**P value**
LA_{major}/Ao	2.1 (1.9-2.3)	1.9 (1.7-2)	0.034
EDVI	49 (45-72)	43 (24-51)	0.064
LVIDdN	1.6 (1.3-1.6)	1.4 (1.2-1.6)	0.068
SV (cm^3)	21.5 (16.9-32)	19.5 (10.4-34.6)	0.448
CVC_{max} (mm)	8.8 (6.5-10.8)	9.2 (8.1-10.8)	0.358
CVC_{max}/Ao	0.62 (0.48-0.70)	0.65 (0.54-0.73)	0.296
CVC CI (%)	40 (33-46)	32 (23-48)	0.350

All the ultrasonographic variables were tested, using binary logistic regression, as potential discriminators between the dogs in the control and study cohorts. The variables that performed better as discriminators were the EDVI, that achieved significance (P=0.047), and the LVIDdN, which was close to, but did not achieve significance (P=0.07). None of the measurements of the CVC had the ability to discriminate between cohorts. All the discriminators tested are listed in Table 3-5. The attempts to obtain a cut-off value for discrimination between cohorts for the EDVI or the LVIDdN were unsuccessful, as the sensitivity and specificity were not considered clinically acceptable for either of these

variables. For the EDVI, the cut-off value that performed best was a value of 50. With this cut-off value, 47% of the patients would have been correctly classified, with a sensitivity of 42%, and a specificity of 53%. For the LVIDdN a cut-off of 1.3 will classify correctly 39% of the patients, with a sensitivity of 68% and a specificity of 11%. Thus, none of the ultrasonographic measurements was able to accurately differentiate between euvolaemic and potentially hypovolaemic dogs.

Table 3-5. Logistic regressions to identify possible discriminators between the control and study cohorts.

Variable	**Odds ratio**	**95%Confidence interval**	**P-value**
LA_{major}/Ao	0.41	0.063-2.70	0.360
EDVI	0.96	0.93-0.999	0.047
LVIDdN	0.08	0.005-1.25	0.070
SV	0.99	0.95-1.03	0.702
CVC_{max}	1.14	0.91-1.42	0.250
CVC_{max}/Ao	10.99	0.13-932.2	0.290
CVC CI	0.14	0.001-24.4	0.460

.

3.1.2 Study cohort pre-treatment vs post-treatment

After analysing the differences between the control and study cohorts, the study cohort was analysed comparing the values at the time of presentation (pre-treatment) against the values after the administration of IV fluid therapy (post-treatment).

On the physical examination, the categorical variables of the study cohort are included in Table 3-6, which showed that most of the patients moved to the categories considered normal post-treatment. In the continuous variables the CRT (p=0.02) was shorter post-treatment, and the HR (p=0.004), was lower post-treatment (Table 3-7).

Table 3-6. Number of dogs included in each category of the categorical variables pulse quality and mucous membranes (MM) colour in the study cohort pre-treatment, and post-treatment.

Variable	**Categories**	**Study cohort pre-treatment**	**Study cohort post-treatment**
Pulse quality	0	6	16
	1	6	2
	2	5	0
	3	1	0
MM colour	0	3	0
	1	9	15
	2	5	3
	3	1	0

The SBP did not show differences pre, and post-treatment (Table3-7).

In the laboratory tests (Table 3-7), LAC failed to demonstrate a substantial change pre, and post-treatment. However, in the subsequent measurements, all the patients returned to values of < 2.5mol/L. The PCV (p=0.001) and TP (p=0.02) were significantly lower post-treatment (Table 3-7).

Table 3-7. Continuous variables from the physical examination and laboratory data from the study cohort pre-treatment and post-treatment with intravenous fluid therapy presented as median (interquartile range). P value obtained by Wilcoxon signed-rank test.

Variables	**Value pre-treatment**	**Value post-treatment**	**P-value**
CRT (s)	2 (2-3)	2 (2-2)	0.02
HR (rpm)	152 (131-160)	113 (100-132)	0.004
SBP (mmHg)	130 (119-152)	136 (119-143)	0.26
LAC (mmol/L)	1.8 (1.5-2.9)	1.9 (1.5-2.5)	0.64
TP (g/L)	64 (56-67)	60 (55-67)	0.02
PCV (%)	48 (37-55)	44 (35-51)	0.001

The ultrasonographic protocol in the study cohort was performed in a median of 4 (3-4) minutes for the pre-treatment examination and 3 (2-4) minutes for the post-treatment examination. The exploration from the subxiphoid view was subjectively more time consuming than the exploration from the right parasternal window. Thus, dogs that needed immediate intervention lacked some measurements from the subxiphoid window. The SV

was measured in fifteen out of the eighteen patients. The CVC measurements were acquired in sixteen out of the eighteen patients. The dimensions of the left heart chambers (LA_{major}/Ao, EDVI, and LVIDdN) were measured in all of the eighteen patients. In the analysis of the ultrasonographic measurements (Table 3-8), the LA_{major} /Ao (p=0.002), the EDVI (p=0.003), and the LVIDdN (p=0.003), were significantly greater post-treatment. The SV (p=0.003) was significantly higher post-treatment. The CVC_{max} (p=0.003) and the CVC_{max}/Ao ratio (p=0.002) were significantly greater post-treatment. The CVC CI did not show any significant change.

Table 3-8. Ultrasonographic measurements pre-treatment and post-treatment in the study cohort, presented as median (interquartile range). P value obtained by Wilcoxon signed-rank test.

Variables	**Value pre-treatment**	**Value post-treatment**	**P-value**
LA_{major}/Ao	1.9 (1.7-2)	2 (1.8-2.3)	0.002
EDVI	43 (24-51)	54 (34-66)	0.003
LVIDdN	1.4 (1.2-1.6)	1.5 (1.4-1.6)	0.003
SV	19.5 (10.4-34.6)	22 (11.7-39.4)	0.003
CVC_{max} (mm)	9.2 (8.1-10.8)	10.5 (8.6-12.9)	0.003
CVC_{max}/Ao	0.65 (0.54-0.73)	0.72 (0.64-0.86)	0.002
CVC CI (%)	32 (23-48)	33 (27-39)	0.54

Graphs were constructed for individual measurements in the study cohort pre, and post-treatment for the ultrasonographic variables: LA_{major}/Ao (Figure 3-1), EDVI (Figure 3-2), LVIDdN (Figure 3-3), SV (Figure 3-4), CVC_{max} (Figure 3-5), CVC_{max}/Ao (Figure 3-6), CVC CI (Figure 3-7)

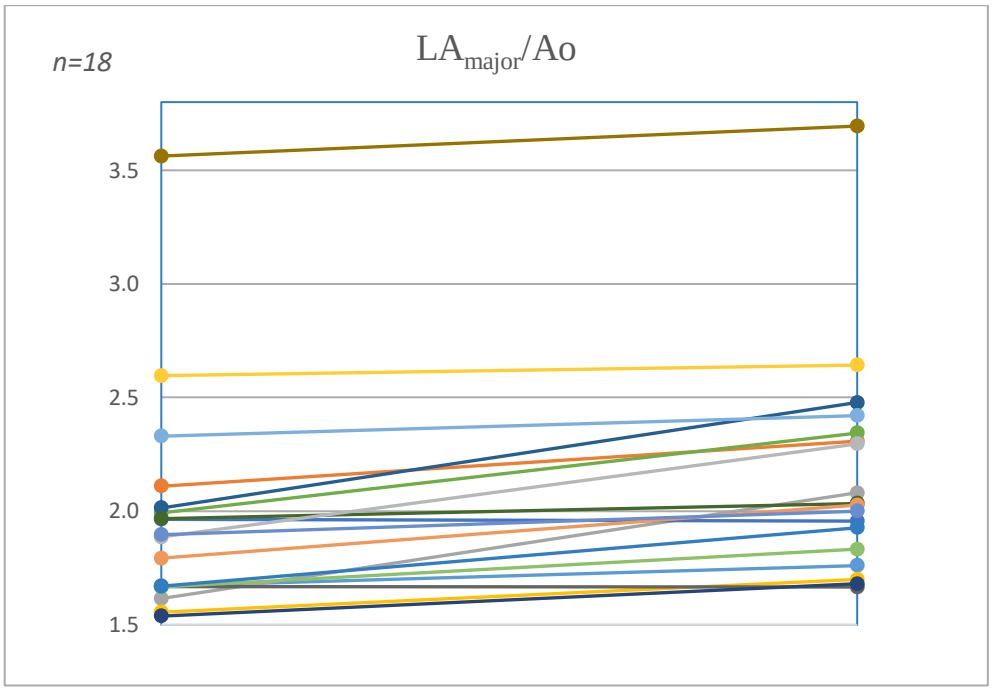

Figure 3-1. Left atrium major diameter to aorta ratio (LA_{major}/Ao) pre- and post-treatment in the study cohort.

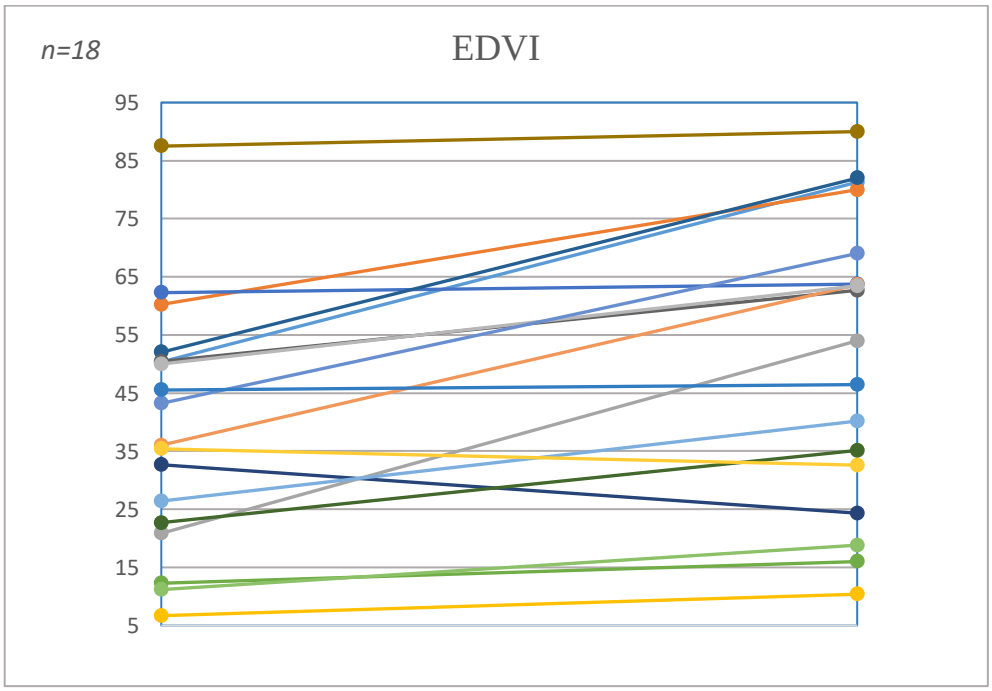

Figure 3-2. End diastolic volume index (EDVI) pre- and post-treatment in the study cohort.

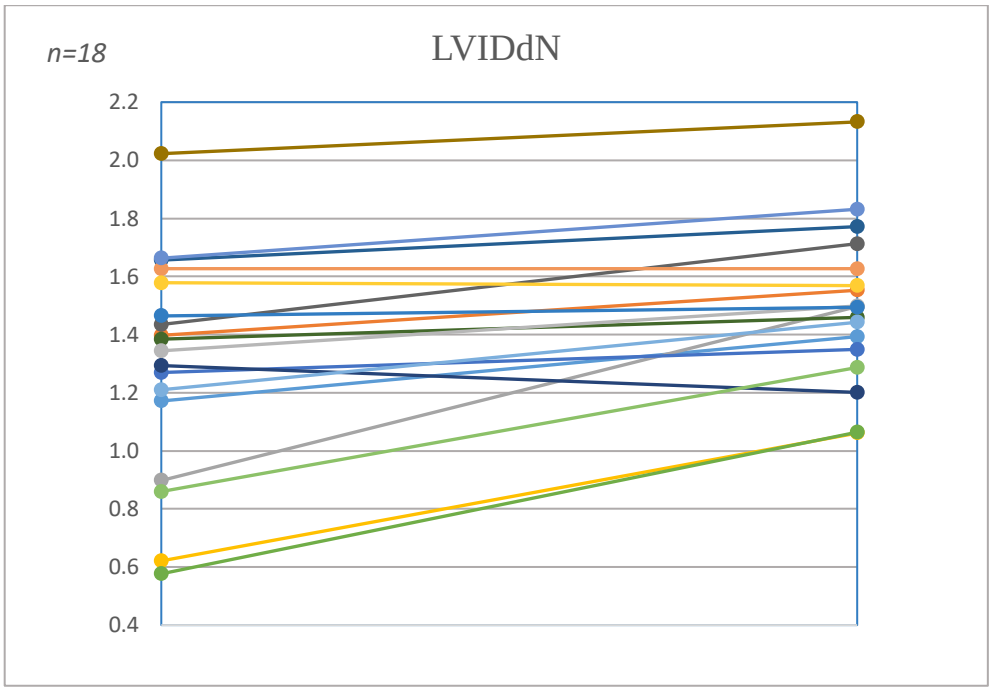

Figure3-3. Left ventricular internal diameter in diastole normalized (LVIDdN) pre- and post-treatment in the study cohort.

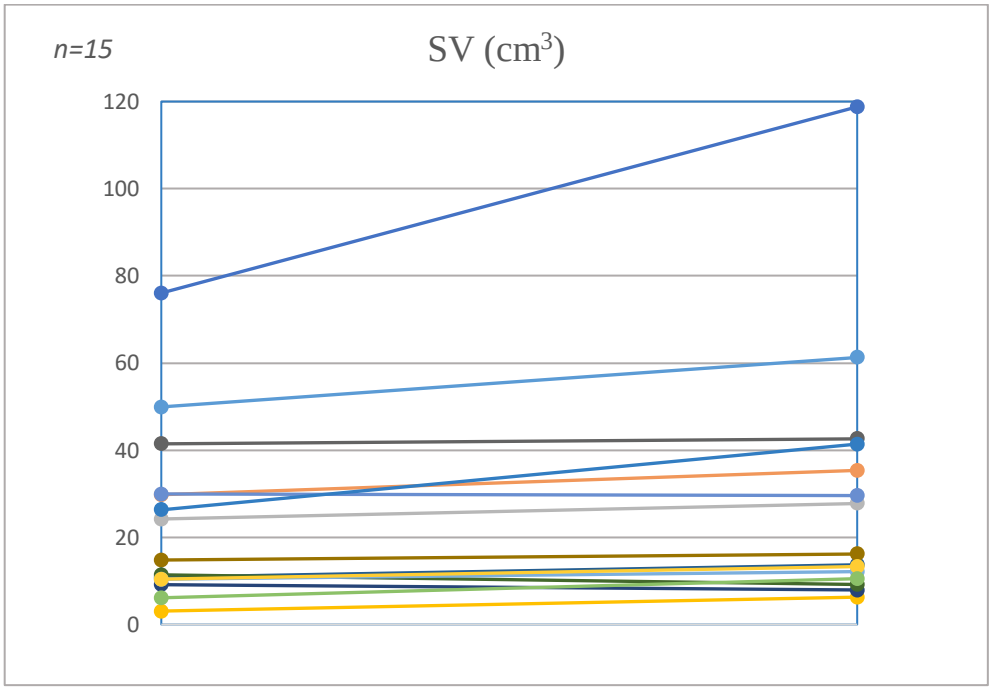

Figure3-4. Stroke volume (SV) in cubic centimetres(cm^3), pre- and post-treatment in the study cohort.

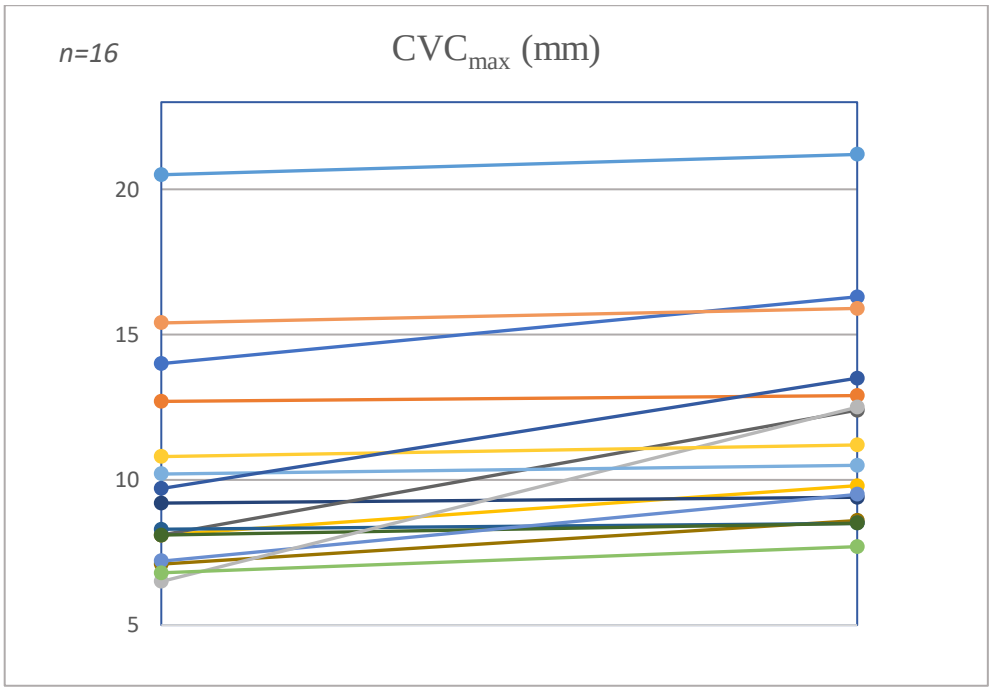

Figure3-5. Caudal vena cava maximal diameter (CVC_{max}) in millimetres (mm), pre- and post-treatment in the study cohort.

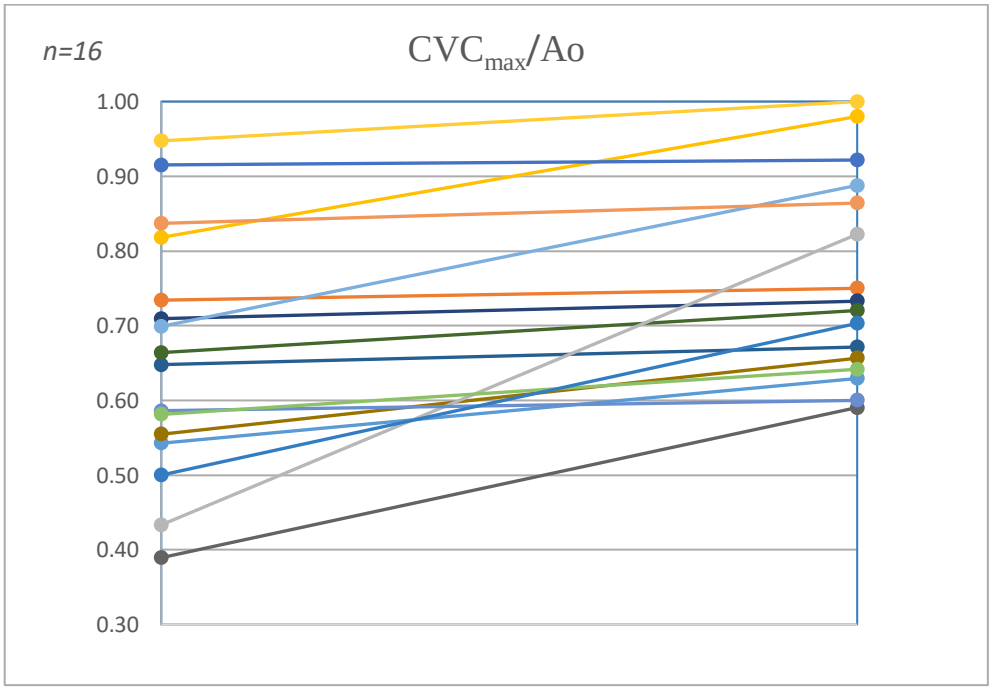

Figure3-6. Caudal vena cava maximal diameter to aorta ratio (CVCmax/Ao) pre- and post-treatment in the study cohort.

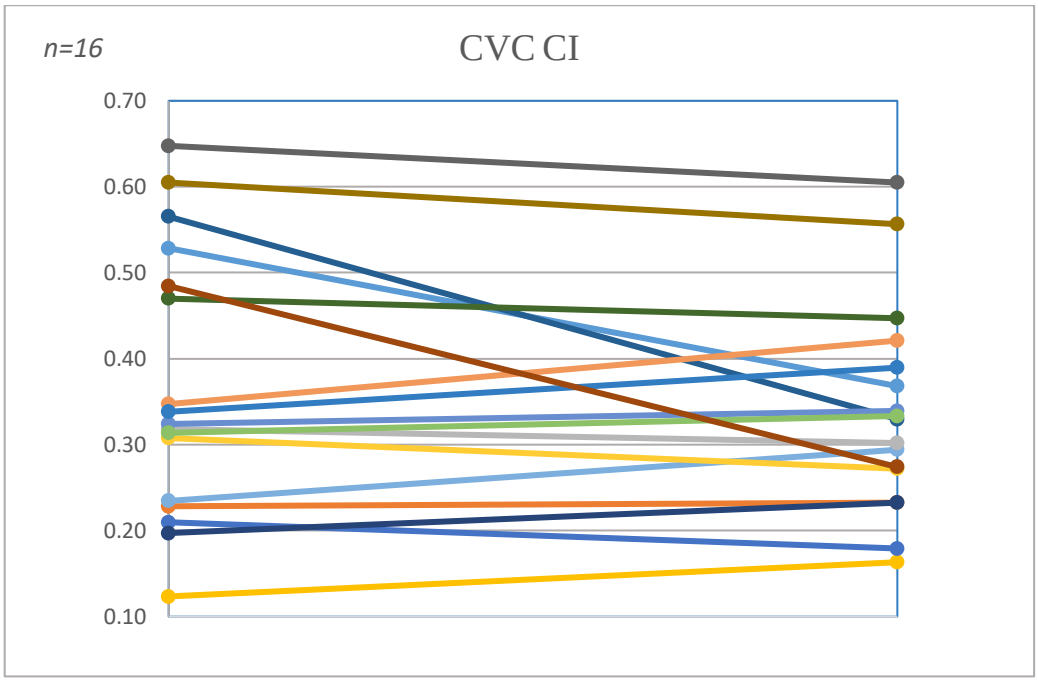

Figure3-7. Caudal vena cava collapsibility index (CVC CI) pre- and post-treatment in the study cohort.

All the patients in the study cohort, except one (patient 9), made a full recovery and were discharged from the hospital.

3.1.3 Fluid responders vs non-fluid responders

Eleven out of the fifteen patients that had SV measurements performed, achieved an increase of 10% or more after the administration of IV fluid therapy, and were classified as fluid responders according to the current standards in human guidelines (Cecconi *et al.*, 2014). Four out of the fifteen patients that had SV measurements performed did not achieve an increase of 10% after the IV fluid therapy and were classified as non-responders (patients 9, 10, 11, 16). Two dogs out of the four non-responders presented with moderate dehydration from gastrointestinal losses (patients 10 and 11). One dog out of the four non-responders presented with chronic kidney failure with gastrointestinal losses, marked polyuria and systemic hypertension (patient 16). The only patient that did not recover was one of the four non-fluid responders (patient 9). This dog did not achieve a 10% increase in SV after fluid therapy, despite the fact that its LAC reduced to <2.5 mmol/L. This patient was euthanised due to a suspicion of a gastric tumour.

The differences in the ultrasonographic measurements between responders and non-responders were analysed (Table 3-9).

Table 3-9. Ultrasonographic measurements pre-treatment and post-treatment in the fluid-responders and non-fluid responders among the study cohort, presented as median (interquartile range). P value obtained by U Mann-Whitney test.

Variables	**Value pre-treatment**		**P-value**	**Value post-treatment**		**P-value**
	Fluid responders	Non-fluid responders		Fluid responders	Non-fluid responders	
LA_{major}/Ao	1.8 (1.7-2.1)	1.9 (1.6-2.4)	0.957	1.9 (1.7-2.4)	2 (1.8-2.4)	0.416
EDVI	41 (23-51)	47 (30-66)	0.481	55 (29-68)	57 (32-74)	0.786
LVIDdN	1.3 (1.1-1-6)	1.5 (1.3-1.8)	0.143	1.5 (1.3-1.6)	1.6 (1.4-2)	0.357
CVC_{max} (mm)	9.5 (7.1-14-3)	8.2 (7.8-10.3)	0.625	11.9 (9.5-16)	8.5 (8.4-11)	0.074
CVC_{max}/Ao	0.67 (0.53-0.86)	0.57 (0.5-0.68)	0.303	0.89 (0.66-0.94)	0.63 (0.54-0.72)	0.034
CVC CI (%)	33 (28-49)	40 (22-61)	0.786	32 (25-37)	40 (28-57)	0.212

.

There were no differences in the pre-treatment values for the LA_{major}/Ao, EDVI, LVIDdN, CVC_{max}, CVC/Ao, CVC CI between fluid responders and non-fluid responders. The post-treatment values were greater (p=0.034) for the CVC_{max}/Ao ratio in the fluid responders. The other variables were not significantly different, although the CVC_{max} almost achieved significance (P=0.074).

3.1.4 Shock vs non-shock hypovolaemia

Seven out of the eighteen dogs of the study cohort were suspected to have hypovolaemic shock based on their physical examination and laboratory tests, whereas eleven out of eighteen dogs were suspected of varying degrees of hypovolaemia without achieving the shock status.

Two of the seven dogs suspected of hypovolaemic shock had intra-abdominal haemorrhage secondary to splenic rupture (patients 3 and 6). Four of the seven dogs suspected of hypovolaemic shock presented with severe dehydration, two of them from gastrointestinal losses (patients 14 and 18), one of them from gastrointestinal losses added to poor cardiac performance secondary to end-stage myxomatous mitral valve disease (MMVD) (patient 9), and another one from severe iatrogenic dehydration from parenteral furosemide treatment (patient 4). The dog suffering from iatrogenic dehydration was also diagnosed with pulmonary hypertension (PHT) secondary to a lung atelectasis.

One of the seven dogs suspected to be in shock was diagnosed with either neurogenic shock or relative hypovolaemic shock due to systemic vasodilation, along with severe bradycardia. The clinical distinction of these two shock entities can be rather challenging, particularly if the cause is a severe blunt trauma, potentially affecting the head, or the upper spinal cord, as it was in this case (patient 2). Trauma can cause mayor bleeding from bigger vessels, which will be detectable clinically as a haemorrhage, or from the capillary bed, which will be clinically undetectable. This bleeding may lead to hypovolaemic shock. Trauma can also cause a disruption in the vasomotor centre, causing a haemodynamic triad of vasodilation, bradycardia and hypotension that characterizes neurogenic shock (Ahuja *et al.*, 2018). In human medicine, it is thought that the distinction between hypovolaemic and neurogenic shock in trauma patients, although theoretically possible, can be virtually impossible clinically, and the recommendation in these patients is to restore intravascular volume. This patient was the only one of the seven patients suspected of shock that had a LAC<2.5 mmol/L, having LAC=1.9mmol/L, at the time of presentation.

Three of the dogs suspected of hypovolaemic shock did not have their SV measured, and two of them did not have their CVC measured as they required immediate intervention due to haemorrhagic shock. It was considered unethical to delay a therapeutical intervention in order to acquire data for the study. However, as the exploration from the right parasternal window can be performed very rapidly by the operator who performed the examination and was also employed to rule out a cardiac mass in the cases of splenic masses, the echocardiographic measurements of the heart chambers (LA_{major}, EDVI and LVIDdN) were available for all seven patients.

As described earlier, one of the shock patients had end-stage MMVD and its measurements were excluded from this analysis of sub-groups due to the increased heart size secondary to MMVD. The differences in the echocardiographic measurements of the heart chambers between shock and non-shock patients were analysed (Table 3-10). The LVIDdN was smaller (p=0.046) in the shock patients pre-treatment compared to non-shock patients. There were no differences in the other measurements pre-treatment. The post-treatment values were not significantly different between shock and non-shock patients.

Table 3-10. Ultrasonographic measurements pre-treatment and post-treatment in the shock and non-shock patients among the study cohort, presented as median (interquartile range). P value obtained by U Mann-Whitney test.

Variables	**Value pre-treatment**		**P-value**	**Value post-treatment**		**P-value**
	Shock	Non-shock		Shock	Non-shock	
LA_{major}/Ao	1.8 (1.6-2)	1.9 (1.7-2)	0.270	2.1 (2-2.3)	2 (1.8-2.1)	0.288
EDVI	33 (14-49)	40 (31-51)	0.212	50 (24-61)	57 (35-65)	0.242
LVIDdN	1.1 (0.7-1.4)	1.4 (1.3-1.6)	0.046	1.5 (1.2-1.5)	1.5 (1.4-1.6)	0.227

3.2 Subjective ultrasonographic findings

The subjective assessment of the CVC diameter and LV diameter were performed at the time of examination in the study cohort, pre-treatment, and post-treatment with IV fluid therapy.

3.2.1 Changes in the caudal vena cava

The subjective appearance of the maximal diameter of the CVC and its change in diameter throughout the cardiac cycle was assessed in sixteen of the eighteen dogs of the study cohort. Three dogs showed subjective dilation of the CVC (Figure 3-8) in the pre-treatment examination (patients 4,7 and 15). This dilation of the CVC in a supposedly hypovolaemic dog prompted the suspicion of increased pressure of the right heart. The three dogs with a subjectively dilated CVC were diagnosed with pulmonary hypertension (PHT), based on a pressure gradient (PG)>30mmHg, derived from the tricuspid regurgitation velocity, when a full echocardiographic examination was performed. The CVC remained dilated in all three dogs in the post-treatment examination.

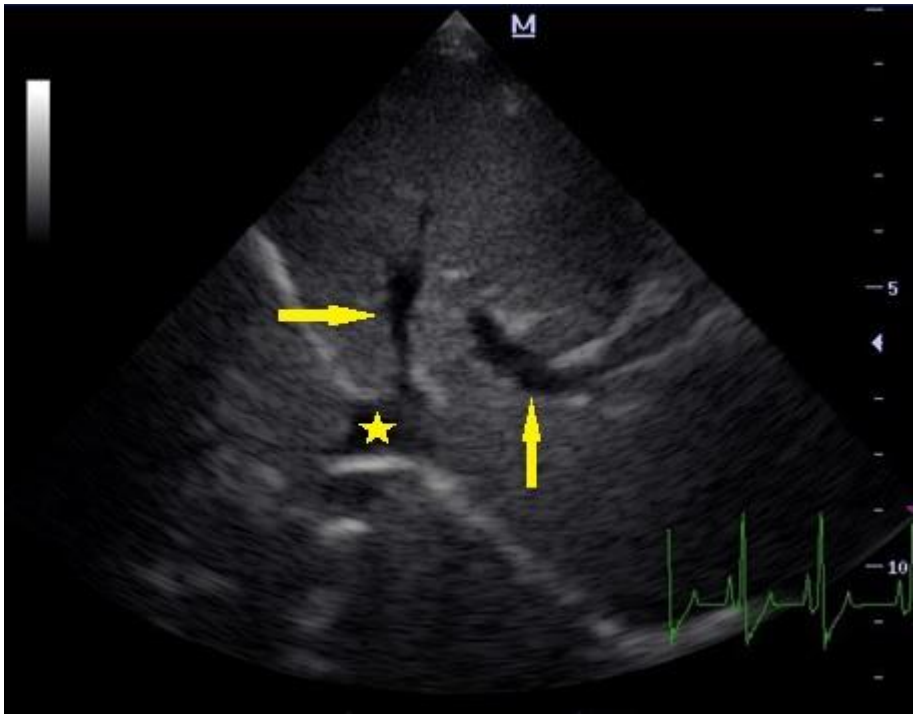

Figure3-8. Longitudinal subxiphoid view showing a dilated caudal vena cava (star) and dilated hepatic veins (arrows) in a dog (patient 15) with pulmonary hypertension and suspected hypovolaemia (pre-treatment).

Eight dogs showed a subjectively normal CVC in the pre-treatment examination, which remained normal in the post-treatment examination. Five dogs showed a subjectively collapsed CVC (patients 8, 12, 14, 17 and 18), with obliteration of the lumen of the vessel at some point during the cardio-respiratory cycle (Figure 3-9) in the pre-treatment examination.

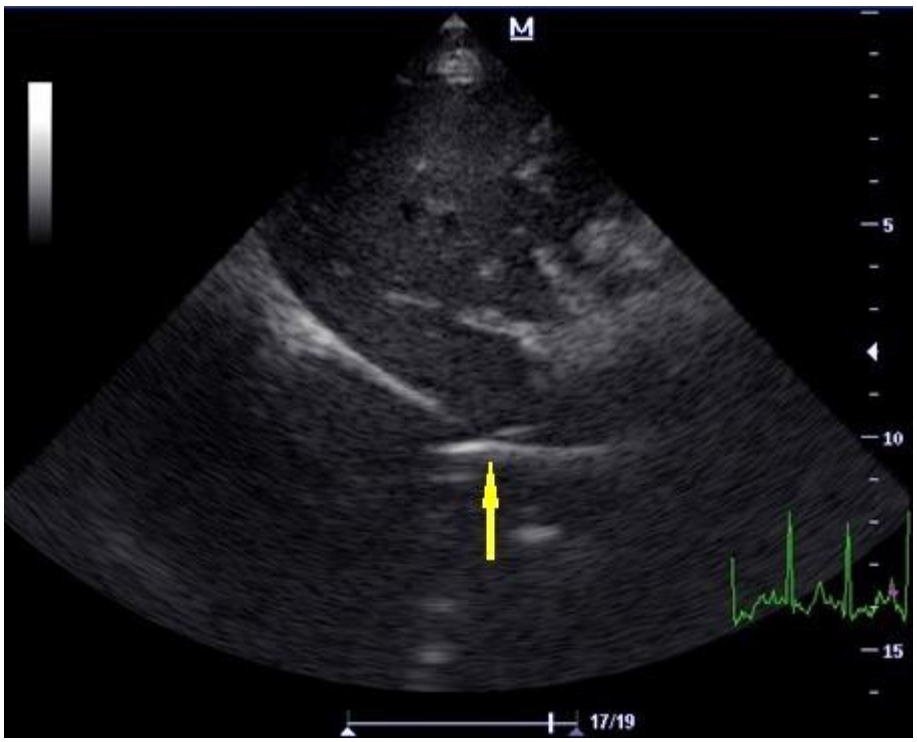

Figure3-9. Longitudinal subxiphoid view, showing complete collapse of the lumen of the caudal vena cava (arrow) in a dog suspected of hypovolaemia (pre-treatment).

The subjective appearance of the CVC changed from collapsed in the pre-treatment examination to normal in the post-treatment examination in the five dogs that had a collapsed CVC pre-treatment (Figure 3-10).

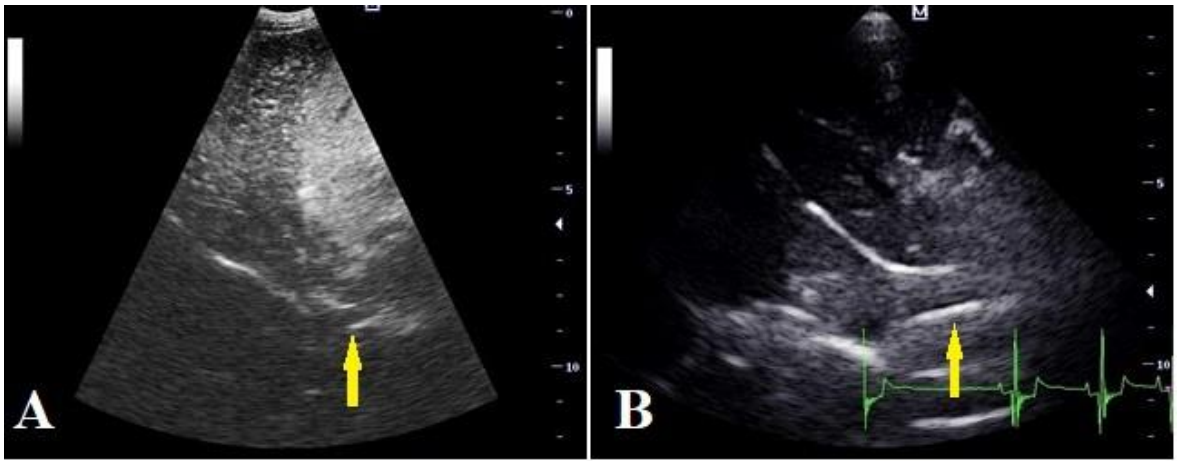

Figure3-10. (A) Maximal diameter of the caudal vena cava from the subxiphoid view (arrow) from patient 14 pre-treatment. (B) Maximal diameter of the vena cava from the subxiphoid view (arrow) from patient 14 post-treatment.

Table 3-11 summarises the number of dogs included in each category for the lumen of the CVC in the pre-treatment and post-treatment examination.

Table 3-11. Number of dogs in each subjective category of caudal vena cava diameter before (pre-treatment) and after (post-treatment) the administration of intravenous fluid therapy.

CVC subjective assessment	**Pre-treatment (n=16)**	**Post-treatment (n=16)**
Dilated	3	3
Normal	8	13
Collapsed	5	0

.

3.2.2 Changes in the heart chambers

The lumen of the LV was assessed subjectively in all eighteen dogs of the study cohort. Three dogs (patients 3, 4 and 6) showed severe obliteration of the left ventricular lumen during systole in the pre-treatment examination (Figure 3-11). It is important to note that the lumen of the left heart chambers, the LA and the LV, was affected unevenly by the phase of the cardiac cycle in the three dogs that showed severe obliteration of the lumen of the LV. Whereas the LV was completely collapsed in systole and showed a visible lumen in diastole, the LA showed subjectively small changes between atrial systole and atrial diastole (Figure 3-12), always keeping a visible lumen.

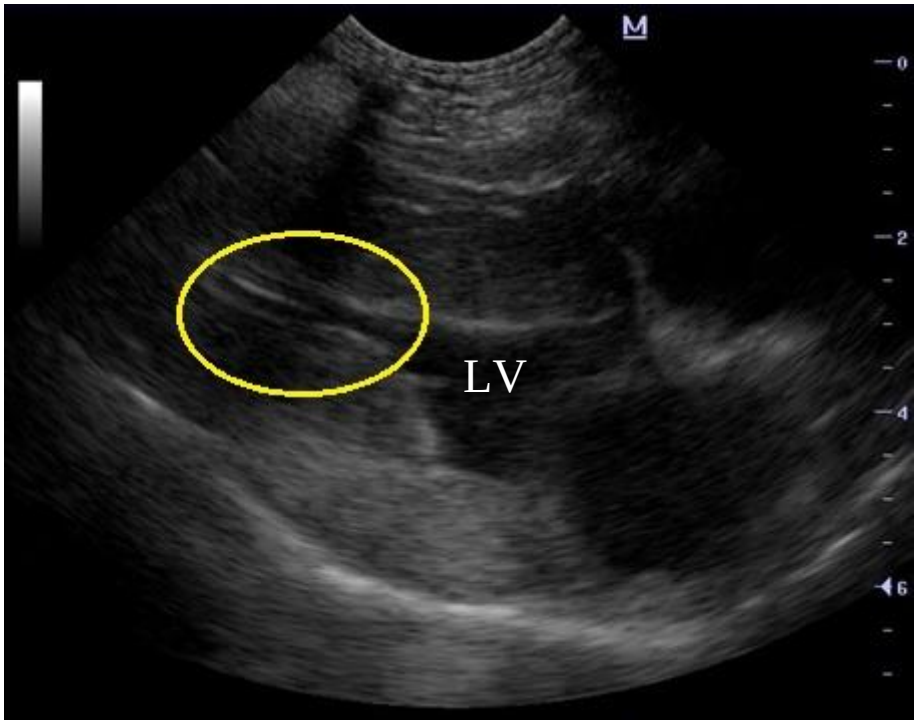

Figure3-11. Right parasternal long axis four chamber view showing severe obliteration of the left ventricular (LV) lumen (encircled area) during systole in a dog suspected of hypovolaemia in the pre-treatment examination.

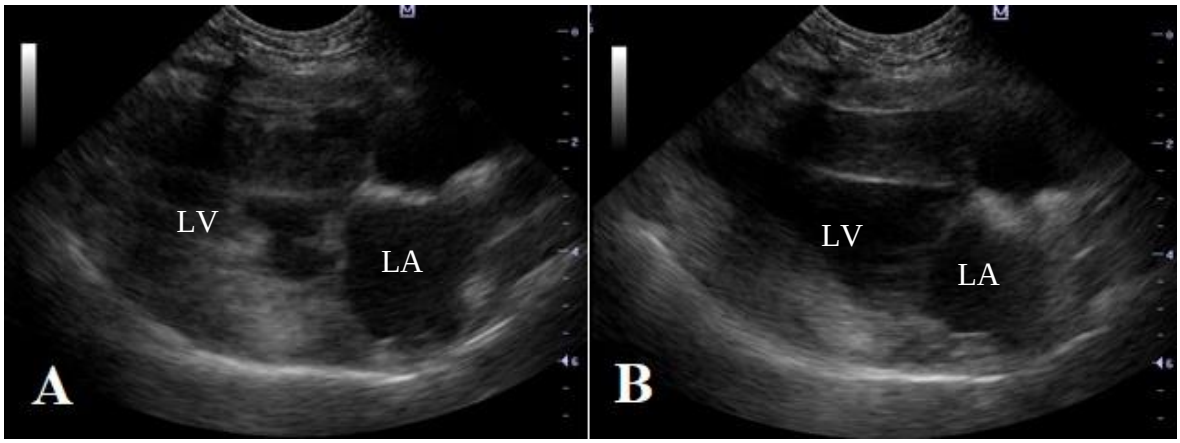

Figure3-12. Right parasternal long axis four chamber view during systole (A) and diastole (B) in the pre-treatment examination of patient 3, showing very marked changes in the lumen of the left ventricle (LV) between systole (A) and diastole (B), while small changes occur in the lumen of the left atrium (LA), which always keeps a visible lumen.

The subjective appearance of the LV changed from severe obliteration in the pre-treatment examination to moderate obliteration in the post-treatment examination in two of the three dogs (patients 4 and 6). This change from severe to moderate obliteration of the LV was observed in both, the L4chA view (Figure 3-13) and the Spm view (Figure 3-14).

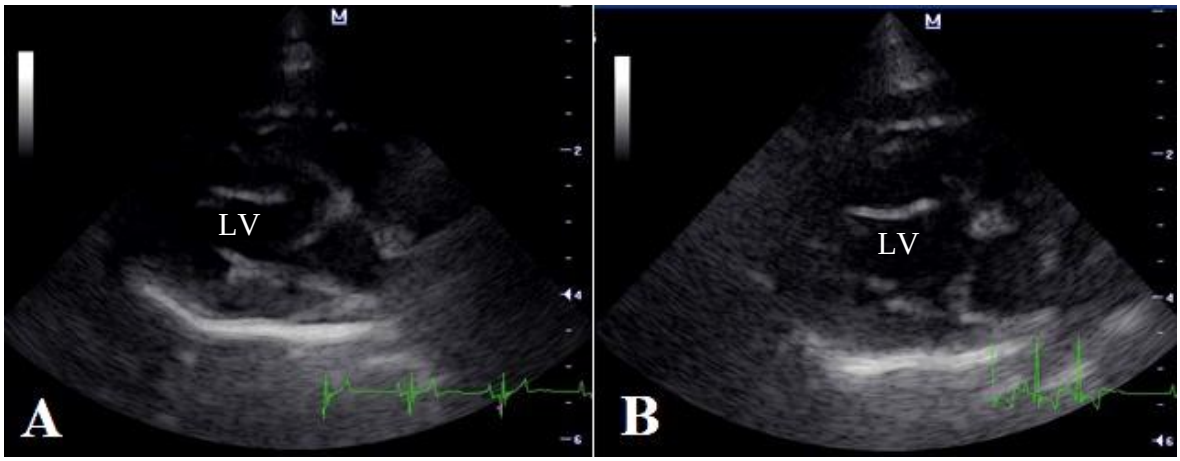

Figure3-13. (A) Right parasternal long axis four chamber view in diastole from patient 4 showing severe obliteration of the left ventricle (LV) pre-treatment. (B) Right parasternal long axis four chamber view in diastole from patient 4 showing moderate obliteration of the left ventricle post-treatment.

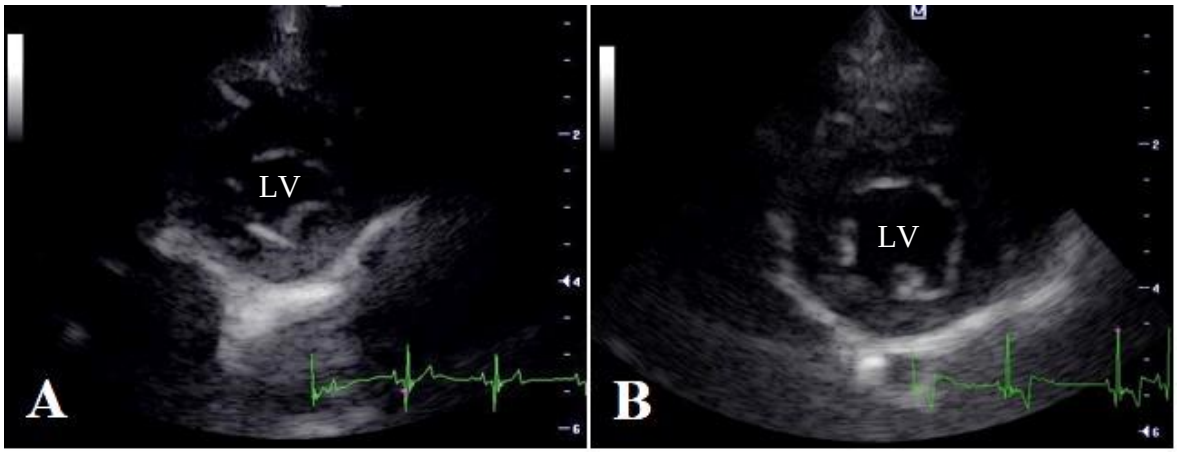

Figure3-14. (A) Short axis view of the left ventricle in diastole from patient 4 showing severe obliteration of the lumen of the left ventricle pre-treatment. (B) Short axis view of the left ventricle (LV) in diastole from patient 4 showing moderate obliteration of the lumen of the left ventricle post-treatment.

One dog (patient 3) changed from severe obliteration of the LV in the pre-treatment examination to no obliteration of the LV in the post-treatment examination (Figure 3-15).

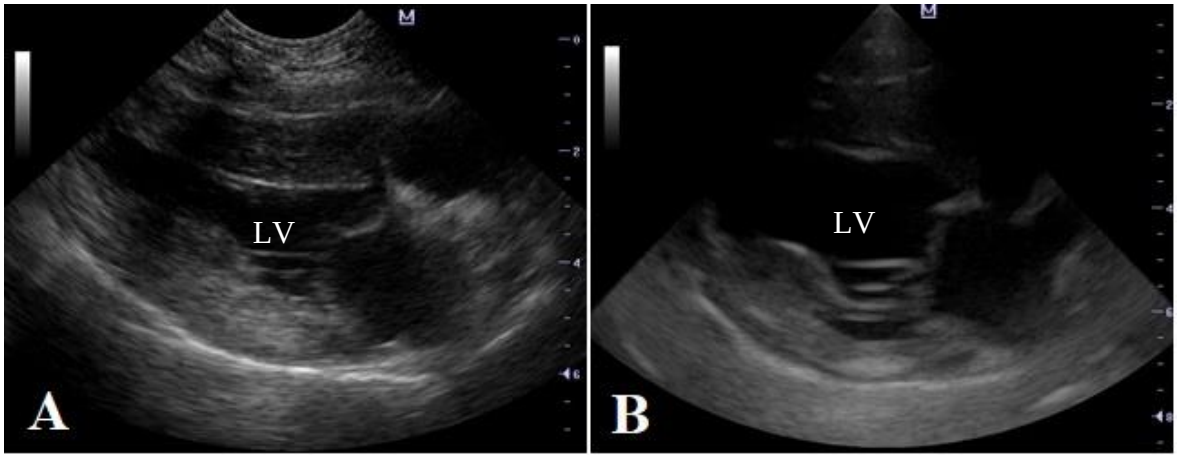

Figure3-15. (A) Right parasternal long axis four chamber view of the left ventricle (LV) in diastole in patient 3 showing severe obliteration of the left ventricular lumen pre-treatment. (B)Right parasternal long axis four chamber view of the left ventricle in diastole in patient 3 showing no obliteration of the left ventricular lumen post-treatment.

Table 3-12 summarises the number of dogs included in each subjective category for the assessment of LV lumen obliteration pre-treatment and post-treatment.

Table 3-12. Number of dogs in each subjective category of left ventricular lumen obliteration before (pre-treatment) and after (post-treatment) the administration of intravenous fluid therapy.

Left ventricular obliteration	**Pre-treatment (n=18)**	**Post-treatment (n=18)**
Severe	3	0
Moderate	6	2
Not present	9	16

3.2.3 Correlation of subjective changes with fluid responsiveness and shock

The correlation of a collapsed CVC or an obliterated LV ventricular lumen in any degree in the pre-treatment examination, with a disruption of tissue perfusion expressed as a LAC≥2.5mmol/L, fluid responsiveness and the presence of shock was investigated, and it is described in Table 3-13. All the five dogs that presented subjectively with a collapsed CVC, also presented some degree of obliteration of the LV lumen and were all positive fluid responders. All the three dogs with severe obliteration of the LV lumen were in shock, and in addition to that they were positive fluid responders.

Table 3-13. Dogs that presented a collapsed CVC or an obliterated lumen of the left ventricle in any degree and the associated presence of an elevated lactate, positive fluid response and shock.

Patient identification	**Collapsed CVC**	**Obliterated lumen LV**	**LAC ≥2.5mmol/L**	**Positive fluid response**	**Shock**
1	no	yes	no	yes	no
3	n/a	yes (severe)	yes	yes	yes
4	no	yes (severe)	yes	yes	yes
6	n/a	yes (severe)	yes	yes	yes
8	yes	yes	no	yes	no
11	no	yes	no	no	no
12	yes	yes	no	yes	no
14	yes	yes	yes	yes	yes
16	no	yes	no	no	no
18	yes	yes	yes	yes	yes

.

4. Discussion

4.1 Changes in the physical examination variables

The assessment of volaemia in dogs is, currently, mostly based on the history and the physical examination (Johnson, 2016). This is due to the lack of the equipment and the training necessary to implement advanced techniques in most veterinary practices. Several variables obtained from the physical examination were included in the current study, proving to be different between the control and study cohorts, and also pre-treatment and post-treatment in the study cohort.

The CRT was different between the control and study cohorts, and also between the pre-treatment examination and the post-treatment examination of the study cohort. The CRT was shorter in the control than in the study cohort at the time of presentation. Also, the CRT was shorter after the administration of fluid therapy to the dogs in the study cohort. However, this shortening may not be of clinical significance, as the median CRT was of 2 seconds for both groups (pre-treatment and post-treatment). In a clinical setting it is unlikely that a clinician would be able to perceive differences of less than a second. Therefore, a prolonged CRT can be informative for the diagnosis of hypovolaemia, but it may not be a useful tool to monitor the response to treatment. In human medicine the CRT is considered as a poor method to estimate the volume status of the patient (Fleming *et al.*, 2015). It is recommended to use a substantially prolonged CRT, over four seconds, just as a *red-flag* sign, to recommend further investigation. In veterinary medicine similar findings have been described, giving the CRT some clinical value to be used as a sign of concern (Boag and Hughes, 2005), and proving it to be inefficacious in estimating the volume status of the dog (Goucher *et al.*, 2019).

The HR was different between the control and study cohorts, and also between the pre-treatment examination and the post-treatment examination of the study cohort. The HR was higher in the study cohort at the time of presentation, than in the control cohort. Also, it was higher in the pre-treatment examination than in the post-treatment examination, after the administration of fluid therapy. When comparing the data from the present study with the reference ranges given in the literature for the assessment of volaemia in dogs (Boag

and Hughes, 2005), the values pre-treatment ,152 (131-160) bpm, fitted in the category of moderate hypovolaemia (150-170bpm), while the values post-treatment, 113 (100-132) bpm, fitted in the category of absence of hypovolaemia (<130bpm) according to the same source. This builds on the current evidence, that the HR is one of the tools clinicians may use to monitor patients suspected of hypovolaemia (Drozdzynska *et al.*, 2018). In this situation, an elevated HR will reflect the activation of the compensatory mechanisms, particularly the increase in sympathetic activity.

There are many other factors that can cause an increase in the HR in emergency patients on presentation, such as stress, pain, hypoxemia, anaemia, or fever (Tilley *et al.*, 2008). These factors can show a response, consistent in a reduction in HR, to other treatments that are not fluid therapy. For instance, an elevated HR caused by pain would respond to the administration of analgesics, if it is caused by stress it would respond to sedatives, hypoxemia would respond to oxygen-therapy, or fever would respond to antipyretics. Many of these drugs, particularly analgesics, were administered to most of the patients included in the study cohort when judged clinically appropriate. Therefore, it is difficult to prove that the reduction in HR was due exclusively to the restoration of the blood volume, but it is also true that the deactivation of the compensatory mechanisms would not occur unless the blood volume is restored. For instance, opioid analgesics will not reduce the heart rate in uncompensated hypovolaemia. According to the present data, and previous evidence (Boag and Hughes, 2005), the HR can be useful in the diagnosis of hypovolaemia, and also in the monitoring of the response to treatment in hypovolaemic dogs (Rabozzi *et al.*, 2020), but it should always be interpreted in the context of the underlying condition and the concurrent administration of other treatments.

The pulse quality was different between the control and the study cohort, which had many dogs in the poorer quality pulse categories. Among the study cohort, the pulse quality seemed to improve post-treatment, as most patients moved to the categories considered normal. However, this is a completely subjective measurement, and can easily include bias when the same clinician is monitoring the treatment and reassessing the patient. Also, even when pulse quality has been described to change mostly accordingly to the SV (Bighamian and Hahn, 2014), it can also be affected by vasoconstriction or vasodilation, phenomena that occur during hypovolaemic states, and also during the recovery from them (Boag and

Hughes, 2005). Therefore, it cannot be concluded that the improvement in pulse quality was due exclusively to the administration of fluid therapy and the restoration of the blood volume. Thus, although pulse quality may be useful in the diagnosis and monitoring of response to treatment of hypovolaemic dogs, it has important limitations, and needs to be interpreted among other variables.

The MM colour showed similar behaviour to the pulse quality. It was different between the control and study cohort, which presented with many dogs in the paler MM colour categories. Among the study cohort, the MM colour seemed to improve post-treatment, as most patients moved to the categories considered normal. MM colour assessment is also affected by similar limitations regarding its subjectivity and the influence of vasoconstriction or vasodilation. Thus, even when in agreement with the expected outcome, it is difficult to prove that the restoration of the blood volume was the cause for the improvement in MM colour. It is likely that the main component for this change in appearance of the MM was the fluctuation in the vasomotor tone (Boag and Hughes, 2005), in response to the activation of the compensatory mechanisms and their deactivation, rather than the changes in the blood volume. We need to mention again that the deactivation of the compensatory mechanisms would not occur unless the blood volume is restored.

Summarising this section, among the physical exam variables, the HR can be helpful in the diagnosis of hypovolaemia in dogs, but it needs to be interpreted in the context of the clinical picture and the underlying disease. Even though, HR can be affected by the administration of other treatments, it seemed to be helpful in monitoring the response to fluid therapy in dogs suspected of hypovolaemia. Other variables such as the MM colour and pulse quality can also be used for the monitoring of treatment but are very subjective and largely influenced by the vascular tone.

4.2 Changes in the systolic blood pressure

Arterial SBP is considered of little value in the assessment of volaemia or fluid responsiveness (Muir *et al.*, 2014). This is due to the high effectiveness of the compensatory mechanisms (RAAS activation, ADH secretion, and increased sympathetic activity) in maintaining a normal arterial SBP during the development of hypovolaemia in conscious dogs.

Hypotension is defined in dogs as a SBP of less than 90mmHg (Silverstein and Hopper, 2014). The changes in the SBP in conscious dogs are mostly related to the vascular tone, and not to the circulating blood volume, unless this is severely reduced. Ideally, the measurement of the SBP should be obtained from invasive measurements from an arterial catheter. The invasive monitoring of the SBP will allow to obtain other parameters, such as the SPV or the PPV, that have shown much better performance to estimate volaemia and fluid responsiveness in mechanically ventilated dogs than the SBP alone (Araos *et al.*, 2020). However, the insertion and maintenance of an arterial catheter in a conscious dog may be challenging (Beal and Hughes, 2000).

In the present study, the SBP was measured by indirect methods, either Doppler or oscillometric. None of these methods meet the requirements for validation (Acierno *et al.*, 2018), although they are considered a clinically acceptable alternative in conscious dogs (Haberman *et al.*, 2006, Bosiack *et al.*, 2010). In the present study, the same method, oscillometric or Doppler, was always used for the same dog for repeated measurements. It is thought Doppler and oscillometric readings are not interchangeable (Wernick *et al.*, 2012). Thus, using the same method to acquire repeated measurements should have reduced the variability of the measurement due to the actual procedure and provide more comparable readings.

Despite the reduction in technical variability, the present study did not prove any statistically significant difference between the pre-treatment and post-treatment readings. As discussed earlier, hypovolaemia and hypotension are not necessarily related. The activation of the RAAS, the secretion of ADH and an increase in sympathetic activity are able to maintain the SBP in a range that should guarantee peripheral perfusion in conscious

dogs. Thus, animals with occult hypovolaemia are not expected to present with hypotension. Hypotension induced by hypovolaemia in conscious dogs will only happen in the decompensated stage of shock, once the compensatory mechanisms have been completely overwhelmed.

In the study cohort, only two dogs had a non-invasive SBP<90mmHg on presentation. One of them had suffered from severe blunt trauma, presumably affecting the head or the upper spine, and was diagnosed with relative hypovolaemia due to systemic vasodilation. In addition to that this dog had a HR of 40 beats per minute, which will substantially reduce the CO. The SBP is directly correlated with the CO and the vascular resistance. Thus, a dog with a reduced CO, due to bradycardia, and a reduced vascular resistance due to vasodilation will be expected to be hypotensive, regardless of the circulating blood volume. The failure to mount an appropriate compensatory tachycardia in this patient may indicate that there was damage to the vasomotor centre and neurogenic shock. In neurogenic shock there is a disruption of the sympathetic activity, with preserved parasympathetic activity (Ahuja *et al.*, 2018). This translates clinically in bradycardia accompanied by vasodilation, leading to hypotension. Whether this dog was affected by neurogenic shock, hypovolaemic shock, or a combination of the two can be difficult to establish. This distinction is described as very challenging and virtually impossible clinically in human medicine, where the recommendation for such trauma patients is to restore intravascular volume, and if this would not be effective, to administer vasopressors, such as dopamine, which was not necessary in this case. This presentation and response to treatment, suggest, once again, that the vasomotor tone, and not the blood volume, was the main cause for the hypotension.

The other dog that had a SBP <90mmHg presented in hypovolaemic shock due to a splenic rupture. This patient was thought to be in the decompensated stage of hypovolaemic shock, with a very high LAC (11mmol/L) and massive intraabdominal bleeding. In addition to the administration of crystalloids and colloids for resuscitation, this patient received a blood transfusion.

The other five dogs diagnosed with hypovolaemic shock, based on their physical examination parameters and LAC, did not show hypotension, and thus they were thought to be in earlier stages of shock. None of the eleven dogs suspected of occult hypovolaemia presented with hypotension.

The administration of fluid therapy did not produce significant changes in the SBP in the study cohort. The medians, pre-treatment and post-treatment were within the normal range of systolic SBP in dogs. According to the data of the present study the non-invasive SBP was of no use for the diagnosis of hypovolaemia, nor for the monitoring of treatment, or to predict fluid responsiveness, as it has been described in the previous literature (Muir *et al.*, 2014, Rabozzi *et al.*, 2020). However, it may be helpful in determining the stage of shock. Patients in decompensated stages of shock will usually present with hypotension, and they may not recover after fluid replacement alone. Thus, additional therapies, such as a blood transfusion may be needed (DiBartola, 2012).

4.3 Changes in the laboratory variables

Additional testing using laboratory variables for emergency patients suspected of hypovolaemia is common practice in veterinary medicine (Johnson, 2016). The PCV and the TP are among the most frequently used.

4.3.1 Changes in the packed cell volume and the total protein

In the present study comparisons between control and study cohort could not be made because of lack of laboratory data in control dogs. In the study cohort the PCV and the TP were lower post-treatment. As most of the patients presented with dehydration, mostly associated with severe gastrointestinal signs, it was expected they were haemoconcentrated on presentation. Haemoconcentration will elevate the values of both PCV and TP. The administration of fluid therapy in a hemoconcentrated animal should increase the circulating blood volume, diluting the cells and plasma proteins, thus causing a reduction in PCV and TP (Davis *et al.*, 2013).

The rapid administration of large volumes of crystalloids in dogs has been proven to be a cause of haemodilution, reducing the PCV and the TP (Valverde *et al.*, 2012). The data from the study cohort builds on the evidence in the literature that serial measurement of the PCV and the TP can be useful in the assessment of fluid volume status in dogs (Silverstein *et al.*, 2005, Davis *et al.*, 2013). However, to evaluate the trend over time of the PCV and the TP, it is crucial that there is complete certainty about the absence of any internal or external bleeding.

The loss of cells and proteins through haemorrhage will also decrease the values of the PCV and the TP. In this scenario, a decrease in the PCV and TP might be erroneously considered the result of a successful blood volume restoration, when in fact, it is depicting a haemorrhage.

Two of the patients included in the study cohort presented massive intraabdominal haemorrhage due to a splenic rupture. They received intensive resuscitative fluid therapy with crystalloids and colloids, followed by a blood transfusion. The blood from the

abdomen was aseptically collected and reinfused in the patient (autotransfusion) as described elsewhere (Robinson *et al.*, 2016). All these interventions would affect the values of the PCV and the TP. While the administration of large volumes of crystalloids will dilute the blood and cause reductions in both, PCV and TP, the administration of an autotransfusion will potentially elevate them. However, the increase in PCV and TP might be asymmetrical, as the blood that has been stored in the abdomen can have a variable content of protein. This is due to the fact that the blood stored in a body cavity will lose, over time, coagulation factors and fibrinogen, as they get consumed by the coagulation cascade that gets activated by being in contact with the inner lining of that body cavity.

The two dogs that received an autotransfusion showed decreased values post-treatment for the PCV and the TP, but this was mostly a consequence of the blood loss, which was not completely replenished even with the blood transfusion. Unexpectedly, the decrease in the PCV was much more marked than in the TP. This may reflect the production of acute phase proteins in response to the hypovolaemic shock. It is possible to identify acute phase proteins in plasma, although it requires advanced laboratory equipment, not frequently available at most veterinary practices. Considering all the confounding factors in the interpretation of the changes in the PCV and the TP, a complete physical examination should be performed along with the measurement of the PCV and the TP each time these parameters are reassessed.

The physical examination should be able to rule out the presence of haemorrhage, and also to assess the changes in the HR, MM colour, and pulse quality. The HR should show a tendency to decrease if the blood volume is being restored (Boag and Hughes, 2005). On the contrary, the HR will increase if the cause for the decreased values of the PCV and the TP was haemorrhage. This increase in HR will be a compensatory mechanism triggered by the reduced amount of oxygen delivered to the tissues by blood containing less oxygen carrying cells, which are being lost by the haemorrhage. Additionally, in case of haemorrhage there will be changes in pulse quality and MM colour. Haemorrhage will make the pulse weaker and the MM colour paler, whereas fluid restoration will make the pulse stronger and the MM colour pinker.

There are currently no guidelines in veterinary medicine to use the PCV and the TP with precision in cases of hypovolaemia, and its use is limited to qualitative changes over time, or in response to therapy, as it was performed in the present study. The necessity for the physical examination parameters to be assessed simultaneously reinforces the idea that none of the laboratory or physical examination parameters can be used individually, and they need to be combined to draw a general picture of the patient, before any decision is made about the volume status of the dog.

4.3.2 Changes in lactate

An elevated LAC might indicate a disruption in tissue perfusion, and it has been traditionally used to identify shock (Cecconi *et al.*, 2014, Gillespie *et al.*, 2017). In the study cohort LAC was not significantly different pre-treatment and post-treatment. In addition to that, it was higher post-treatment in eight of the eighteen dogs.

The dogs diagnosed with hypovolaemic shock, had the highest LAC, and six of the seven had LAC≥2.5mmol/L. Four of the seven dogs in shock showed a marked reduction in LAC after resuscitative fluid therapy, while three of them showed higher values after the fluid bolus, even though the physical exam parameters and the ultrasonographic values had improved. When these three patients were followed for a longer period, and additional therapies implemented, the subsequent LAC measurements were lower, and fell within the reference range (Sharkey and Wellman, 2013).

The failure to normalize LAC and acid-base disorders despite a normalization of the physical exam after resuscitative fluid therapy in sick dogs has been described before, proposing that hypoxemia at the cellular level persisted despite the normalization of the macrocirculation (Young *et al.*, 2014). The administration of lactate-containing crystalloid solutions has been identified as a cause for exacerbation of hyperlactataemia in dogs with lymphoma (Vail *et al.*, 1990). All the dogs included in the study cohort received lactate-containing crystalloid solutions, and it is possible this may have played a role, even though this might have been of small magnitude. Elevated sympathetic activity, which is one of the compensatory mechanisms against hypovolemia, induces hyperlactataemia (Levy *et al.*,

2008). A failure to control additional causes of epinephrine secretion, such as pain, stress, fear, or other components of the shock process, apart from hypovolaemia, may have contributed to the persistently elevated values of some patients.

The three patients in shock that did have increased LAC levels after fluid therapy (patients 2,4 and 18), presented additional causes for hyperlactataemia.

Patient 2 had suffered a severe blunt trauma and it is possible than the muscle damage and the adrenergic response related to it contributed to an elevated LAC more than the relative hypovolaemia, particularly considering that this animal was the only one of the seven dogs diagnosed with shock that had LAC<2.5mmol/L on presentation.

Patient 4 presented with hypovolaemic shock from iatrogenic volume depletion (unnecessary aggressive IV furosemide treatment), and complete atelectasis of one lung with concurrent PHT. Several factors could have exacerbated the hyperlactataemia, such as local hypoxia of the affected lung, the obstructive component of the shock related with the PHT, and the ventilation/perfusion mismatch in the lungs, causing generalized hypoxemia. Additional treatment of this patient with oxygen-therapy and a pulmonary vasodilator (sildenafil) resulted in the normalization of LAC.

Patient 18 had an intestinal obstruction and was in hypovolaemic shock due to extreme dehydration. Local hypoxia is a cause for type A hyperlactataemia; thus, it is likely that the intestinal hypoxia added to the adrenergic activation due to the pain from the intestinal obstruction caused this persistent elevation of LAC. Hyperlactataemia in this dog resolved immediately after performing corrective surgery.

In addition to these additional factors that may have increased LAC, previous publications have identified a window of six hours for LAC to reduce in critically ill veterinary patients (Stevenson *et al.*, 2007). As most of the patients in the present study were reassessed before six hours, and all the LAC measurements came back to normal in the subsequent measurements, it is likely that the changes in the physical exam, PCV, TP, and ultrasonographic measurements preceded the clearance of LAC. The fact that LAC may need several hours to clear might reduce its usefulness to guide fluid therapy in dogs in shock, although it was proven as a useful endpoint for therapy.

The dogs that were not diagnosed with shock but instead, were suspected of having varying degrees of hypovolaemia, had LAC within the reference range. This was expected, as LAC is a late indicator of hypoperfusion (Gillespie *et al.*, 2017), and would only be increased during hypovolaemia when the compensatory mechanisms are overwhelmed, in the decompensated stages of shock. The effect of fluid therapy on the LAC of the eleven patients suspected of varying degrees of hypovolaemia was unpredictable. Six dogs showed reductions, while four showed increases, and one remained the same. The LAC always remained within the reference range for the eleven dogs.

There are two physiological explanations for the erratic behaviour of LAC in these dogs: the timing of the measurement and the movement of fluids through the body compartments.

Occult hypovolaemia comprises a wide range of haemodynamic situations, depending on the degree of hypovolaemia and the degree of activation of the compensatory mechanisms. LAC has roughly a linear relationship with hypoperfusion in dogs, once the compensatory mechanisms have been overwhelmed (Gillespie *et al.*, 2017). However, before this happens, the balance between blood volume deficit and activation/deactivation of the compensatory mechanisms can disrupt this relationship. Depending on the rate of metabolism and clearance of lactate by the liver and kidneys, and on that balance of activation/deactivation of the compensatory mechanisms, fluctuations in LAC in either direction would be expected, and the timing for the measurement will largely affect its value.

Another explanation is the movement of fluids through compartments. Lactate is generated inside the cells, so it will initially accumulate in the intracellular compartment, generating a concentration gradient between the intracellular and interstitial compartments. During dehydration, there is a reduction in the content of fluids in the extracellular compartment, which will then be compensated by a shunting of fluid from the intracellular to the

extracellular compartment (interstitial and intravascular). The lactate molecules will leave the cell following the concentration gradient and be present now in the interstitial space. Crystalloids administered in the intravascular compartment are rapidly redistributed to the interstitial compartment, “washing” the lactate present in this compartment, and thus, transiently increasing intravascular LAC, but maintaining it always within the reference range. The four dogs out of the eleven suspected of occult hypovolaemia that had higher LAC post-treatment (patients 8, 13, 15 and 16) were dehydrated on presentation, making this second mechanism of balance between compartments, suitable. Thus, LAC was unable to inform about the volume status of the patient or monitor the response to treatment in dogs with occult hypovolaemia. LAC was useful for the diagnosis of shock, although even in these patients it was unable to monitor the response to treatment in the short term and needed several hours to normalize.

Summarizing, the laboratory variables are not suited for the diagnosis of hypovolaemia. Although LAC is used for the diagnosis of shock, it is insensitive in diagnosing occult hypovolaemia. The changes in LAC in response to therapy seemed to be delayed when compared with other parameters such as PCV, TP, the ultrasonographic measurements and the physical exam variables. The PCV and TP can be used to monitor the response to treatment in non-blood loss hypovolaemia.

4.4 Changes in the caudal vena cava measurements

The ultrasonographic measurement of the IVC to assess the volume status of the patient and predict fluid responsiveness is a common technique in human medicine (Zhang *et al.*, 2014). However, its clinical usefulness is still under debate and the latest review and meta-analysis concluded that it is an unreliable means of predicting fluid-responsiveness in the general human population (Orso *et al.*, 2020). The main limitations found in this meta-analysis were the vast heterogenicity in the protocol for evaluation of the IVC between studies, and the different methods used to estimate fluid responsiveness or volume status to compare against the IVC measurements. Despite this, it remains in use, because it has been proven useful to guide fluid therapy in some subpopulations of human patients, such as spontaneously breathing critically ill patients (Corl *et al.*, 2017), mechanically ventilated people (Dipti *et al.*, 2012), or those in septic shock (Feissel *et al.*, 2004), acute circulatory failure (Zhang *et al.*, 2014), or haemodialysis (Mandelbaum and Ritz, 1996). In addition to that, measurement of the IVC has been found to improve outcome when used to guide fluid therapy in human intensive care units (Bernier-Jean *et al.*, 2017).

The low cost and rapid execution of the ultrasonographic assessment of the CVC has drawn the attention of veterinary medicine in the recent years (Boysen and Lisciandro, 2013). The same limitations described in the human literature affect the research performed in this field in veterinary medicine: there is a vast heterogenicity in the ultrasonographic protocol used to acquire the measurements, and there is also heterogenicity in the means to estimate volume status.

4.4.1 Technical considerations in the assessment of the CVC in dogs.

In the present study the CVC_{max} was measured in 2D mode from the long-axis view of the CVC from the subxiphoid window, which is considered the optimal view in humans (Finnerty *et al.*, 2017) and has already been tested in dogs (Kwak *et al.*, 2018, Darnis *et al.*, 2018) and validated in horses (Tuplin *et al.*, 2017). However, when reference values for the CVC in dogs were published (Darnis *et al.*, 2018), this view was found to be less repeatable than other approaches and there were no reference values reported for it, but only a mean ± standard deviation of the measurements.

It was decided to use the long-axis view of the CVC from the subxiphoid window in the present study to reduce the examination time, as this is the same ultrasonographic window used to obtain the most accurate Doppler spectrum of the aortic forward flow (Abbott and Maclean, 2003) needed to estimate the SV and define fluid responsiveness. This allowed the two measurements to be obtained from the same window, therefore substantially reducing the time needed to perform the ultrasonographic examination. Also, the subxiphoid view was employed because it has been shown that a training course of only six hours allowed non-cardiologist veterinarians to acquire values that agreed with those obtained by veterinary cardiologists when measuring the CVC_{max} (Darnis *et al.*, 2019) from this window. As the ultimate goal of this research project was to provide a cage-side tool for non-cardiologists, it was considered appropriate to use techniques that are easy to perform and quick to learn.

The positioning of the dog is another factor that needs to be considered when interpreting the results. The dogs in the present study were placed in right lateral recumbency, which is the standard positioning employed to acquire the echocardiographic views that were used in the study (L4chA, L4chV, and Spm) (Thomas *et al.*, 1993), and also the positioning used in the study that validated the CVC/Ao ratio in dogs (Cambournac *et al.*, 2018). However, most publications to date in veterinary medicine, have used the left lateral recumbency (Bucci *et al.*, 2017, Darnis *et al.*, 2018, Kwak *et al.*, 2018, Marshall *et al.*, 2018, Donati *et al.*, 2020, Rabozzi *et al.*, 2020).

The different recumbencies have been proven to affect the measurements of the vena cava in human medicine (Mookadam *et al.*, 2011), and it is likely that this is also the case in veterinary medicine. Thus, the results of the present study may not be completely comparable with previous studies in dogs. In addition to that, there are disparities between publications in using the long axis view, or the short axis view of the CVC.

In the present study the long axis view was used as described in human medicine and in the first description of the method in veterinary medicine (Tuplin *et al.*, 2017). However more recent publications (Darnis *et al.*, 2018) argue that it can be challenging to ensure that the diameter that is being visualized is the maximum diameter of the CVC when it is explored in the long axis. In this view, only transecting the CVC at its centre, will provide the real

maximum diameter, and any obliquity may affect the measurement. Thus, the long axis subxiphoid view of the CVC may be suboptimal to quantitatively assess the CVC in dogs.

The inaccuracy in obtaining the maximal CVC diameter might be avoided if the short axis view is used, and it has been described that the short and long axis measurements of the CVC are not interchangeable (Darnis *et al.*, 2018). That study also described that the CVC in the short axis view often times presented an elliptical shape, so instead of a diameter, the area of the CVC should be used, making the measurements more time consuming.

Reference values for the CVC obtained from echocardiography, from the left parasternal cranial view, have been published in dogs (Gentile-Solomon and Abbott, 2016). This view has not been tested against other views, or in diseased animals, but it may be of use if echocardiographic measurements are employed to estimate volaemia. One limitation to this approach would be the need to manipulate the patient to move it from the right lateral recumbency, where all the other echocardiographic views would be obtained, to the left lateral recumbency to measure the vena cava. Although, this is usually the case when performing a full echocardiographic examination.

Due to the vast morphological heterogenicity of the canine population, the CVC_{max} was normalised to the aortic diameter to obtain a value that was independent of body weight. This method was first described in children (Kosiak *et al.*, 2008), and has been validated in healthy dogs subject to a blood donation (Cambournac *et al.*, 2018). Also it was employed in healthy dogs administered IV furosemide or deprived from water to induce hypovolaemia (Kwak *et al.*, 2018), and most recently in an heterogeneous population of hospitalized conscious dogs (Rabozzi *et al.*, 2020). However, the diameter of the aorta was measured in different locations and through different views in the different studies.

In summary, there is an urgent need to develop a standard ultrasonographic protocol to quantitively assess the CVC in dogs to avoid the same difficulties described in human medicine about the lack of standardization.

4.4.2 Agreement of the CVC measurements with the previous literature

The dogs in the control cohort showed values of the CVC_{max} within the range provided for the same view in the only study in veterinary medicine that attempted to provide reference values for the CVC (Darnis *et al.*, 2018). A more recent study (Vientós-Plotts *et al.*, 2019) established a threshold to describe CVC dilation in dogs weighing less than 9kg or more than 9kg. All the dogs in the study cohort of the present study were under the appropriate threshold, showing agreement between the present study and the previous literature.

Unlike the $CVC_{max,}$ the CVC_{max} /Ao ratio of the control dogs did not fall within the range of measurements given by Darnis *et al.*, 2018, and were lower in the present study. This can be explained by the different methodology employed. While Darnis *et al.* 2018 measured the diameter of the abdominal aorta, in the present study this artery was measured between the aortic valve hinges in the L5ch view. It would be expected that this would be the maximal diameter of the aorta, while the abdominal aorta might have a smaller diameter. A bigger diameter for the aorta would therefore generate a lower value for the CVC_{max} /Ao ratio, as noted.

The range for the CVC CI in the control cohort of the present study mostly coincided with the range provided by Darnis *et al.* 2018, although the median, and third quartile were higher than theirs. It has been described that the range of normality for the CVC CI is very wide in healthy human individuals (Finnerty *et al.*, 2017) and in healthy dogs (Darnis *et al.*, 2018), so the lack of complete agreement possibly reflects the different characteristics of the population included in the different studies. Another explanation may be the difficulty to ensure proper alignment when measuring the CVC_{min}, needed to calculate the CVC CI. In a previous study in dogs, the CVC_{min} measurements showed much poorer agreement between cardiologists and non-cardiologists than the measurements of the CVC_{max} (Darnis *et al.*, 2019). Thus, inaccurate measurement of one of the components of the index, will prevent the actual index to be reliable.

The generally good agreement with previous publications suggests the technique may be repeatable and comparable between observers if a standard protocol is followed.

4.4.3 Quantitative changes in the CVC measurements

The CVC_{max} and CVC_{max}/Ao were unable to discriminate between the healthy controls and the dogs suspected of hypovolaemia in the present study. The same finding has been described in another very recent study in spontaneously breathing dogs (Donati *et al.*, 2020). The explanation for this can be in the wide range of normality for these parameters. Even when there is a change in the diameter for a given animal, this can still fall within the range of normality. Also, there are several factors other than the blood volume that can affect the diameter of the CVC, such as the intraabdominal pressure, the intrathoracic pressure, and the diaphragmatic excursion. Donati *et al*., 2020 concluded that the assessment of the diameter of the CVC without accounting for the respiratory cycle is not an accurate predictor of fluid responsiveness, as described in humans (Airapetian *et al.*, 2015).

According to the results of the present study, both CVC_{max} and CVC_{max}/Ao were greater after the administration of fluid therapy in the study cohort. This builds on other evidence in the literature that the fluid volume status affects the diameter of the CVC in dogs (Cambournac *et al.*, 2018 Kwak *et al.*, 2018, Donati *et al.*, 2020, Rabozzi *et al.*, 2020). These authors demonstrated reductions in CVC_{max} and CVC_{max}/Ao after blood loss (Cambournac *et al.*, 2018), volume depletion or dehydration (Kwak *et al.*, 2018), in other words the CVC reduced its diameter in response to blood volume contraction. In the present study increases in CVC_{max} and CVC_{max}/Ao were documented when fluid therapy (blood volume expansion) was administered to dogs suspected of hypovolaemia. This is in agreement with the findings of a recent study, which studied a very similar population (Rabozzi *et al.*, 2020). This suggests the assessment of the CVC_{max} and CVC_{max}/Ao may be useful to monitor the response to treatment in dogs suspected of hypovolaemia.

When the dogs in the study cohort that were fluid responders were compared to those that were non-fluid responders there were significant differences in the CVC_{max}/Ao in the post-treatment values between the two groups. Therefore, the ability of a dog to achieve an increase in the CVC_{max}/Ao demonstrated a positive fluid response, which, again, agrees with the findings of Rabozzi *et al*. 2020. However, since only eleven of the dogs in the study group were proven to respond to fluid therapy, these observations may lack statistical power.

Previous studies in veterinary medicine have described a wide range of normality for the CVC CI in healthy animals (Tuplin *et al.*, 2017, Darnis *et al.*, 2018) but have hypothesized that a narrower range would be seen in pathologic states (hyper and hypovolaemia), a finding that has already been reported in human patients (Finnerty *et al.*, 2017). Darnis *et al.* 2018 suggested the CVC CI will be very low in hypervolaemia and very high in hypovolaemia, as seen in humans (Stawicki *et al.*, 2009). According to the data in the present study the CVC CI was unable to discriminate between controls and clinical cases, as it was to monitor the response to treatment.

A poor capacity of the CVC CI to assess volume status has already been reported in humans (Gui *et al.*, 2018) and dogs (Marshall *et al.*, 2018, Rabozzi *et al.*, 2020). In a study with healthy human volunteers that performed a passive leg raising test, although the CVC CI was slightly lower after the test, this difference was so small and inconsistent that it was judged of little value in the clinical setting (Gui *et al.*, 2018). Similar findings were reported in dogs subject to a blood donation. Even though the CVC CI was slightly higher after 8% blood loss, the change was of such a small magnitude that it would probably not be helpful in clinical patients.

However, another human study showed good agreement between CVC CI and other techniques (echocardiography and bioreactance) in predicting fluid responsiveness in spontaneously breathing clinically ill human patients, although it identified a different threshold for positive fluid response than previous studies (Corl *et al.*, 2017). Also, a very recent study in dogs (Donati *et al.*, 2020), showed that the CVC CI was predictive of fluid responsiveness in spontaneously breathing dogs with perfusion abnormalities. They explained how the cardiac cycle and the respiratory cycle can affect the venous return and the intrathoracic pressure respectively, explaining why some results obtained in mechanically ventilated dogs (Bucci *et al.*, 2017), cannot be applied to spontaneously breathing dogs. They concluded that the absolute diameter of the CVC may be of no use, if it does not take into consideration the respiratory variations (Donati *et al.*, 2020).

Thus, further research is required in both human and veterinary medicine to reach a conclusion about the usefulness of this index in clinical patients to assess volume status.

4.4.4 Additional indications for the CVC measurements

The measurements of the CVC may provide information about other variables that are not the volume status. The first proposed use for the measurement of the CVC in dogs presented as emergencies was to raise a suspicion of increased pressures in the right side of the heart if the vessel was dilated (Lisciandro, 2011, Boysen and Lisciandro, 2013). It should be noted that three dogs in the present study (patients 4, 7 and 15) diagnosed with PHT, and thus, with elevated right atrial pressure, presented with a subjectively dilated CVC, even during hypovolaemic shock for one of them (patient 4).

However, a more recent study declared the CVC_{max} to be insensitive when screening for PHT (Vientós-Plotts *et al.*, 2019). This was counterintuitive and against the initial hypothesis of that study. They hypothesized that the heterogenicity of the operators acquiring the images, and factors such as respiratory movements, or the pressure applied with the ultrasonographic probe may have affected the measurements. Only one operator performed all the measurements in the present study, excluding the inter-operator variability as a source of error.

Of the three dogs diagnosed with PHT, patient 15 was diagnosed with hypovolaemia secondary to dehydration from gastrointestinal losses, and showed modest changes in the heart chambers size, and almost no change on the CVC measurements after fluid therapy, although it was classified as a positive fluid responder (SV increase >10%) and had a very noticeable clinical improvement after fluid therapy. This dog had the greatest CVC_{max}/Ao ratio, with a value of 1 after fluid therapy, of all the dogs included in the study (control and study cohorts). However, when compared with a recent study that established a cut-off value to describe a CVC as dilated, this dog did not reach this threshold (Vientós-Plotts *et al.*, 2019). This may be a consequence of the ranges of weight provided by that study and the lack of normalization to any other blood vessel. They stablished that an absolute diameter of the CVC obtained from the subxiphoid view over 1.4 cm for any dog weighing more than 9kg and over 0.9 cm for any dog weighing less than 9kg was indicative of caval dilation. As the canine population is very heterogeneous it is likely that a measurement normalised to the aorta would be more sensitive to describe such changes.

Patient 4 presented with iatrogenic hypovolaemic shock as a consequence of unnecessary aggressive furosemide treatment and showed dramatic increases in the left heart chambers sizes, but a modest increase in the CVC after fluid therapy. This dog showed the lowest value of CVC CI (16% after fluid therapy), and the second biggest CVC_{max}/Ao ratio (0.98 after fluid therapy) of all the dogs included in the study. The concurrent observation of a distended vena cava and a low CVC CI has been described as a reliable indicator of elevated right atrial pressure in human patients (De Vecchis *et al.*, 2016) and seems to prove true in this dog that was diagnosed with severe PHT. When tested against the cut-off value provided by Vientós-Plotts *et al*. 2019, it was proven to have a dilated CVC after fluid resuscitation, but not before, when in hypovolaemic shock. This dog was a positive fluid responder based on its SV variation after fluid therapy.

The last of these three dogs (patient 7) diagnosed with PHT had MMVD and was suspected of hypovolaemia as a consequence of iatrogenic Addisonian crisis secondary to treatment with trilostane. It also was a positive fluid responder according to the SV variation, in spite of minimal changes in the heart chamber sizes and the CVC measurements, which showed CVC dilation before and after treatment according to the cut-off values from Vientós-Plotts *et al*. 2019.

The increases in the CVC_{max} and CVC_{max}/Ao ratio in all these three patients after fluid therapy were very modest, suggesting that the presence of elevated right atrial pressure can be a limitation to the CVC measurements to assess volume status if used alone.

The usefulness of the CVC CI to monitor for increased pressures in the right atrium has already been assessed in human patients (De Vecchis *et al.*, 2016). Using the CVC CI in conjunction with the CVC diameter, and using a threshold of CVC CI <50% and CVC_{max} >21mm, was predictive of increased right atrial pressure. Two of the three dogs of the present study (patients 4 and 15) diagnosed with PHT had CVC CI<50%, and their CVC_{max}/Ao ratios were the first and second highest of all the patients in the study. Also two of the three (patients 4 and 7) were over the threshold of caval dilation for dogs (Vientós-Plotts *et al.*, 2019). This suggests that similar features can be seen in dogs than in humans. However, further research would be needed to establish appropriate thresholds.

Considering all the limitations and variables, when faced with a dilated CVC, it will be impossible, without echocardiography, to determine if the patient is hypervolemic or suffers from elevated right atrial pressure due to PHT or right sided congestive heart failure. Thus, the quantitative CVC measurements and the CVC CI should always be interpreted in conjunction with echocardiography.

In summary, according to the findings of the current study, the CVC measurements may not be suitable for the diagnosis of hypovolaemia in dogs. However, the CVC_{max} and the CVC_{max}/Ao ratio may be useful in monitoring the response to fluid therapy and establishing fluid responsiveness. They can also rise a suspicion of elevated right atrial pressures, which can be further assessed by the use of echocardiography.

4.4.5 Subjective changes in the CVC

The subjective appearance of the CVC can also be assessed by ultrasonography. In the study cohort of the present study, the diameter of the CVC subjectively increased after the administration of IV fluid therapy. In addition to that, the presence of a collapsed CVC seemed to predict positive fluid responsiveness, as all the dogs with a collapsed CVC at presentation were positive fluid responders and showed a normal size CVC after the administration of fluid therapy. This should be interpreted with caution due to the small sample size, only 5 dogs. However, the presence of a collapsed CVC is very easy to diagnose, and reassess by the same or different clinicians (Darnis *et al.*, 2019), and may become a simple way of guiding fluid therapy.

4.5 Changes in echocardiographic measurements.

Echocardiography is the standard of care for human patients in shock (Cecconi *et al.*, 2014) and a useful tool in the monitoring of blood volume status in critically ill human patients (McLean, 2016). There are recognised limitations for the measurements of the CO using echocardiography in humans, where it has been proven to not be interchangeable with the readings obtained from thermodilution (Wetterslev *et al.*, 2016). However, the estimation is considered clinically acceptable, and the information that the technique can provide is so clinically relevant, that it can successfully guide interventions of paramount relevance such as fluid therapy (Porter *et al.*, 2015, Boyd *et al.*, 2016, Miller *et al.*, 2016).

In veterinary medicine there is very scarce literature about the usefulness of echocardiography as a guide to fluid therapy, or in the management of shock. Most of the studies to date were aimed at estimating the CO during anaesthesia or to develop animal models for human medicine. The transoesophageal modality has shown excellent agreement with thermodilution, which is considered the gold-standard (Yamashita *et al.*, 2007, Mantovani *et al.*, 2017) proving that the Doppler flow profile is capable of estimating CO accurately. However, this technique is obviously unsuited for conscious patients, and the equipment required is seldom available in veterinary practices. The accuracy of transthoracic echocardiography to estimate CO in dogs is still under debate. While some authors have suggested clinically acceptable agreement with the invasive readings from thermodilution (Lopes *et al.*, 2010) or from a flowmeter (Uemura *et al.*, 2013), other authors have demonstrated poor agreement (Day *et al.*, 2007).

The present study is the first attempt in veterinary medicine to use echocardiographic measurements to assess volume status in conscious, spontaneously breathing, clinically ill dogs suspected of hypovolaemia. Previous studies have demonstrated a reduction in the cardiac chambers measured by transthoracic echocardiography when healthy dogs (Fine *et al.*, 2010), horses (Underwood *et al.*, 2011) or cats (Sugimoto *et al.*, 2019, Campbell and Kittleson, 2007) were experimentally dehydrated by water deprivation or volume depleted by the administration of furosemide. The present study agrees with these publications in the sense that changes in the volume status produced measurable changes in the cardiac chambers.

4.5.1 Selected echocardiographic variables for the assessment of volaemia.

The left atrium was measured using its maximal diameter in the L4chA view. Although the left atrium to aorta ratio (LA/Ao) has been traditionally used for the estimation of the left atrial size, the maximal diameter of the LA in the long axis view has been demonstrated to outperform this ratio, being more accurate in estimating the real size of the atrium and also more repeatable (Strohm *et al.*, 2018). In addition to that, the L4chA view has been traditionally used to assess the heart size and function subjectively and using this same view to acquire atrial measurements will reduce the time required to perform the examination. Also, the L4chV, just represents a slightly different optimization for the same view, allowing the acquisition of two views to acquire measurements of the LA and the LV in a very short time. These measurements were acquired in right lateral recumbency, which is the standard for echocardiography in dogs (Thomas *et al.*, 1993). The position of the dog affects the echocardiographic measurements of the heart (Chetboul *et al.*, 2005), as it does with the CVC.

The LV was measured in two different ways: the left ventricular size was measured using the LVIDdN, and the left ventricular volume was estimated using the EDVI obtained by the Simpson's method of discs. The LVIDdN is considered one of the most repeatable echocardiographic measurements in terms of low inter day, inter observer and intra observer variability (Dukes-McEwan *et al.*, 2002, Visser *et al.*, 2019). In the author´s opinion, the Spm view that is needed to measure the LVIDdN is one of the easiest echocardiographic views to obtain and optimize for measurement. It has been shown that a course of only six hours is enough for non-cardiologist veterinarians to be able to accurately acquire the end diastolic left ventricular diameter (Darnis *et al.*, 2019) used to calculate the LVIDdN. In addition to the simplicity of the acquisition of the view, the calculation of the LVIDdN does not require special cardiac software, widening the range of ultrasonographic equipment that can be employed. Another advantage of the LVIDdN is that being a linear measurement it minimizes the influence of any operator-related error when compared with a volumetric measurement such as EDVI, where any operator-related error during the measurement acquisition would be amplified to the power of three when the volume is calculated.

The EDVI obtained by the Simpson´s method of discs is currently considered the optimal measurement to identify subtle changes in the left ventricular volume (Visser *et al.*, 2019), which justifies why it is used in the diagnosis of occult phases of heart diseases that show LV volume overload, such as dilated cardiomyopathy (Wess *et al.*, 2010). The left parasternal apical four chamber view and the L4chV view which was used in the present study, have been proven equally valid for the acquisition of the LV volume by the Simpson´s method of discs (Wess *et al.*, 2010). The inter and intra observer repeatability of the EDVI is very good among veterinary cardiologists (Wess *et al.*, 2010), but this has not been tested in non-cardiologists. In the author´s opinion, obtaining an optimized L4chV view and repeatable measurements for the EDVI is much more technically challenging than acquiring the end diastolic left ventricular diameter used for the calculation of the LVIDdN. In addition to that, it requires special cardiac software to calculate the volume after the left ventricular area has been traced. However, if very subtle increases in the EDVI reveal early volume load, it would be expected that small reductions in the EDVI may demonstrate early volume depletion.

The SV estimated by transthoracic Doppler echocardiography has been described to show clinically acceptable agreement with thermodilution in anaesthetised dogs (Lopes *et al.*, 2010), although that study showed better performance for the values obtained from the pulmonary artery than for those obtained from the aorta, classifying the aortic SV as not clinically acceptable. This is probably a consequence of better alignment with the pulmonary flow, than with the aortic flow, as the accuracy of the Doppler profile is angle-dependant (Thomas *et al.*, 1993). A poorer alignment with the aortic flow could have been a consequence of the echocardiographic view they employed, as the left parasternal four chamber apical view they used is considered inferior to the subxiphoid view for the acquisition of the aortic flow (Abbott and Maclean, 2003).

Other studies (Yamashita *et al.*, 2007, Mantovani *et al.*, 2017), which employed transoesophageal echocardiography, demonstrated that the SV calculated from the aortic profile had good agreement with thermodilution, suggesting that a properly aligned aortic flow can accurately estimate SV in dogs. The subxiphoid view was used in the present study to acquire the aortic flow, which could have potentially provided a more accurate estimation of the SV than the one obtained by Lopes et al. 2010. However, this could not

be proven as no other technique, such as thermodilution, was used to validate the estimated SV. The SV estimated by echocardiography was also chosen because it is the standard of care in human medicine (McLean, 2016).

4.5.2 Quantitative changes in the echocardiographic measurements

The dogs in the control cohort showed values among the reference values provided in the literature for the LVIDdN (Cornell *et al.*, 2004, Visser *et al.*, 2019), EDVI (Visser *et al.*, 2019) and LA_{major}/Ao (Strohm *et al.*, 2018). In contrast with the measurements of the CVC, echocardiographic views and reference values are very strictly standardised, helping in the interpretation of the results.

When the echocardiographic measurements of the study cohort were tested against the control cohort, the only discriminator that achieved significance was the EDVI. This might prove that EDVI is able to identify subtle changes in the LV volume, as it was anticipated, and could be a suitable method to detect volume depletion in conscious, spontaneously breathing, clinically ill dogs. However, when a cut-off value was tested, no value demonstrated acceptable levels of sensitivity and specificity. It is possible that a bigger sample size for both cohorts would have yielded a better discriminator and allowed the definition of a cut-off value, so further research in this field is warranted. The LVIDdN was the second-best discriminator, but it did not achieve statistical significance. As for the EDVI it is possible that the study was underpowered, and a bigger sample size would have provided better performance. Although, it can be said from the results of the present study that the ventricular measurements performed better than the atrial and caval measurements, which were unable to discriminate between healthy dogs and those suspected of hypovolaemia.

According to the data of the present study the administration of IV fluid therapy induced changes in the echocardiographic parameters in the study cohort. The size of the left cardiac chambers (LA_{major}/Ao and LVIDdN), the volume of the LV (EDVI), and the SV were greater after the administration of IV fluid therapy in conscious, spontaneously breathing, clinically ill dogs suspected of hypovolaemia.

The fact that the blood volume status affects the size of the cardiac chambers in echocardiography is in agreement with previous veterinary literature in healthy dogs (Fine *et al.*, 2010), cats (Campbell and Kittleson, 2007) and horses (Underwood *et al.*, 2011). All these three studies demonstrated that a reduction in preload results in a reduction of the diameter of the LV, and its volume, and also that it modifies the intracardiac pressures, affecting the systolic and diastolic function in dogs (Fine *et al.*, 2010) and cats (Sugimoto *et al.*, 2019). Therefore, these authors demonstrated a reduction in cardiac size as a consequence of volume depletion, while the present study demonstrated an increase in cardiac size as a result of blood volume expansion. Although the interactions between the cardiac function and the compensatory mechanisms that are activated during hypovolaemia (sympathetic activity, ADH release and RAAS activation) can be very complex, with varying degrees of peripheral resistance, vascular tone, and intravascular pressure, it is clear from the results of the present study and the previous literature that the changes in preload affect the cardiac size. Thus, the LA_{major}/Ao, the LVIDdN and the EDVI may be of use in monitoring the response to treatment in conscious, spontaneously breathing clinically ill dogs receiving IV fluid therapy.

However, it should be noted that pre-existing volume loading cardiac diseases such as MMVD, dilated cardiomyopathy, patent ductus arteriosus (PDA) or ventricular septal defect (VSD) will compromise the diagnostic ability of left chamber sizes and volumes in the estimation of volaemia. Two of the dogs included in the study cohort had advanced MMVD and had significantly enlarged left heart chambers. The administration of IV fluids in these two patients elicited a measurable increase in LA_{major}, EDVI and LVIDdN, with one of them achieving an increase of over 10% in SV (positive fluid responder). The CVC measurements in these two dogs also increased post-treatment and did so in a greater magnitude than the heart chambers, suggesting again that the simultaneous use of the echocardiographic and caval measurements improves their performance.

4.5.2.1 Quantitative changes in fluid responders vs non-fluid responders

The dogs in the study cohort were subdivided into fluid-responders and non-fluid responders according to the impact of fluid therapy on their SV. Human intensive care physicians are familiar with a concept scarcely used in veterinary medicine: fluid responsiveness. A positive fluid responder would be a patient in shock who will benefit from the administration of IV fluids, whereas a negative fluid responder will require a different strategy, and IV fluids can be either unhelpful or detrimental.

There are different techniques in human medicine to assess fluid responsiveness; one of the most common is measuring the SV before and after the administration of a bolus of IV fluids (McLean, 2016). If the human patient achieves an increase in SV of 10% or more, it is classified as a positive fluid responder (Cecconi *et al.*, 2014). Although the measurements of the CO estimated from transthoracic echocardiography in humans are not interchangeable with the readings obtained from thermodilution (Wetterslev *et al.*, 2016), the good trending ability of the SV estimated by echocardiography and the non-invasive nature of the technique have made echocardiography the standard of care for the estimation of fluid responsiveness in humans (Cecconi *et al.*, 2014).

Eleven of the fifteen dogs in the current study that had their SV measured pre- and post-treatment with IV fluids achieved an increase of that magnitude and were classified as positive fluid responders. The SV and not the CO was used in the statistical analysis to avoid the variability that can occur in the HR as a consequence of other treatments. The estimation of the SV through echocardiography will add another tool to the intensive care clinician in veterinary medicine to monitor the response to treatment of dogs suspected of hypovolaemia and give another step towards goal-directed fluid therapy.

Of the four dogs that did not achieve an increase of 10%, and were classified as non-fluid responders, one had severe systemic hypertension and chronic kidney disease (patient 16), two had mild to moderate dehydration from gastrointestinal losses (patients 10 and 11) and one suffered from hypovolaemic shock (patient 9). The mechanisms that may explain a lack of response are different in each of these patients.

The presence of an increase afterload due to systemic hypertension can affect stroke volume, particularly in a clinically ill dog, with severe uraemia. Systolic dysfunction secondary to uraemia has been recognised in human patients and it is described as uremic cardiomyopathy (Josephs and Odenthal, 1995). Dogs are also suspected to have some form of systolic disfunction associated with uraemia (Pouchelon *et al.*, 2015), and this might have prevented an improvement in the SV after the administration of IV fluid therapy in this dog.

Cardiac function deterioration may also explain the lack of response of the dog in hypovolaemic shock that did not show a positive fluid response. This dog (patient 9) had pre-existing MMVD in stage C (Keene *et al.*, 2019) and showed very enlarged cardiac chambers. A previously dilated LV with poor systolic function may have prevented any response in SV. This dog was the only one among the study cohort that did not recover.

The other two dogs had moderate dehydration, and despite them being classified as negative fluid responders, they showed marked clinical improvement after the administration of fluid therapy. This may be explained by the mechanisms which govern the movement of fluids between the body compartments. Dehydration starts in the interstitial compartment, and it is not until the functional reserve in this compartment has been exhausted, that the blood volume starts to decrease. In this scenario, it is possible that dogs that are not severely dehydrated do not change their blood volume. Thus, the preload will remain constant and there will be no change in the SV after fluid therapy. Then, the crystalloids administered would go to replenish the interstitial compartment instead of expanding the blood volume. This puts into question the reliability of fluid responsiveness as a guide to fluid therapy, as being a non-fluid responder in this case does not mean than the patient would not benefit from fluid therapy. This has been a long-standing controversy in human medicine that remains unresolved (Marik and Lemson, 2014, Monnet *et al.*, 2016a).

4.5.2.2 Quantitative changes in shocked vs non-shocked

The study cohort was subdivided into dogs that presented in shock and dogs with varying degrees of hypovolaemia (non-shocked). The LVIDdN was smaller in the dogs that were in shock. Although this subpopulation only comprised of six animals, and thus obtaining a cut-off value was not attempted, it can be inferred that the LVIDdN could potentially be of use in the diagnosis of hypovolaemic shock. The same limitations of the low power of this small sample apply as for the EDVI in the diagnosis of hypovolaemia. Considering the rest of the findings in the present study and what has been described in the previous literature, it would appear intuitive that the size of the LV would get smaller and smaller as the animal progresses into hypovolaemic shock.

4.5.3 Subjective changes in the heart chambers

Not only measurements, but also a subjective assessment of the heart was made through echocardiography. When assessing the images subjectively, a markedly different response to severe hypovolaemia was identified between the LA and the LV. While the LV showed dramatic changes during systole, with complete collapse of the lumen, a phenomenon called *kissing walls* (Leung and Levine, 1994), the LA did not show very marked changes throughout the cardiac cycle. The collapse of the left ventricular lumen possibly reflects an enhanced forward flow due to a marked decrease in afterload, in the decompensated stage of shock. On the other hand, the decrease in blood volume, and thus in preload will affect similarly both chambers, proved by the fact that they were both reduced in size in their respective end diastoles when compared with the values after treatment. All the dogs that showed kissing walls of the LV suffered from hypovolaemic shock, and this phenomenon was no longer observable after the administration of fluid therapy. This builds on the evidence that the obliteration of the LV lumen is diagnostic of hypovolaemia (Feissel *et al.*, 2001). Many of the dogs in the study cohort showed changes in the left heart chambers after the administration of IV fluid therapy that could be identified subjectively. However, this judgement was made by one single observer who has extensive experience in echocardiography, and this may not be applicable to the general population of veterinarians.

In addition to volume status, the subjective assessment of the heart can provide other haemodynamic information. The subjectively enlarged right chambers of three dogs prompted full echocardiography at a later stage confirming a presumptive diagnosis of PHT. These three dogs also presented with a subjectively dilated CVC, reinforcing the concept that the heart chambers and the CVC should be assessed simultaneously.

4.6 Proposed ultrasonographic protocol for the assessment of volaemia in dogs

The present study would support that an ultrasonographic protocol comprising of echocardiography and caval measurements could potentially be a useful tool to assess the volume status of the conscious, spontaneously breathing, clinically ill dog. This protocol will help in raising a suspicion of hypovolaemia and in guiding fluid therapy. Based on the results of the present study a suggested ultrasonographic protocol would comprise:

- Subxiphoid view to obtain the Doppler spectrum of the aortic flow to estimate SV, and to subjectively assess and measure the CVC.
- L4chV view to subjectively assess the heart chambers size and measure the EDVI.
- Spm view to subjectively assess the LV size and to measure the LVIDdN.

The results of this assessment will help in the management of the conscious, spontaneously breathing, clinically ill dog, as described in Table 4-1.

Table 4-1. Clinical applicability of ultrasonographic variables

Variable assessed	**Clinical relevance**
Subjectively collapsed CVC	suggests it should have a positive fluid response
Subjectively collapsed LV	strongly suggests hypovolaemic shock
Increase in CVC/Ao after fluid therapy	suggests positive fluid response
Increase of 10% in SV	strongly suggests positive fluid response
Dilated CVC and dilated right heart chambers	suggest increased pressure in the right atrium and/or pulmonary circulation

.

4.7 Limitations

There were several limitations in this study. Even though this was a prospective study, not all the patients had all the ultrasonographic measurements recorded. This was due to ethical reasons. The animals that were in severe hypovolaemic shock needed immediate intervention, and that limited the time available to perform the examination. The most time-consuming measurement is the SV, as it requires optimal alignment with the aortic forward flow to produce a reliable measurement, and thus, it was only performed in fifteen of the eighteen dogs. Also, as these were privately owned animals, some repeated laboratory variables were not measured after treatment due to financial reasons.

The number of animals included in each cohort was low, considering the objective of obtaining a reliable cut-off value for the diagnosis of hypovolaemia. According to the power calculations based on previously published data about the caval measurements in conscious, clinically ill dogs, the minimum number of animals to detect a change would have been nineteen patients in each group. That number of dogs was achieved in the control cohort, but there was one dog down in the study cohort.

The same sample size of nineteen dogs was deemed appropriate for the other ultrasonographic variables, but this is not necessarily true. The variances of echocardiographic variables in this particular population of dogs, may be different from the variances of those variables in healthy dogs that are used to generate reference values. The lack of published data about the variance of echocardiographic measurements in heterogeneous populations of conscious, clinically ill dogs, made the power calculations rather challenging and possibly inaccurate, increasing the chances of incurring in type II statistical error, which will reduce the ability of the study to recognise the suitability of the technique to diagnose hypovolaemia.

All the subpopulations that were assessed, such as fluid-responders, or animals in shock, did not achieve the minimum sample size proposed, reducing even further the statistical power of the study, and increasing the chances of a type II statistical error. Bigger populations would have been needed to increase the power of the study.

Another limitation is the lack of an alternative method to validate the estimation of the volume status and the fluid-responsiveness. Although the definition of fluid-responsiveness in humans (Cecconi *et al.*, 2014) was followed, this method is yet to be validated in veterinary medicine, thus there was not complete certainty that the dogs in the study cohort were accurately classified as being hypovolaemic, or positive fluid-responders. Thermodilution would be the gold-standard to prove both, hypovolaemia, and fluid-responsiveness, but this technique is seldom available in veterinary premises. There is currently not a gold-standard for the diagnosis of shock.

A further limitation is the fact that the same clinician assessed the patients and performed the ultrasonographic examination, which can introduce bias, as the operator was not blinded to any of the physical exam parameters, laboratory findings, or response to treatment of the dog.

4.8 Future research considerations

The assessment of volaemia using ultrasonography has gained importance in veterinary medicine in recent years. However, veterinarians are inheriting the same problems that this method has encountered in human medicine. The primary problem being the lack of standardization of the method to acquire the measurements of the CVC. Future research should focus on clearly establish the positioning of the patient, and the ultrasonographic views that should be used for both the subjective and quantitative assessment of the CVC, which has shown promising potential in the guidance of fluid therapy.

The results presented on this thesis suggest that the EDVI could potentially be of use in the diagnosis of hypovolaemia. It is possible that a bigger sample size would have permitted determination of a cut-off value for this measurement in order to diagnose hypovolaemia in dogs, and possibly even in grading it. Further research to obtain a cut-off value for EDVI for the diagnosis of hypovolaemia should be pursued.

As LVIDdN is less sensitive than EDVI, it may not be of use for the diagnosis of varying degrees of hypovolaemia. However, the present results suggest that it may be helpful in the diagnosis of hypovolaemic shock. Non-cardiologist veterinarians acquire competence in obtaining this measurement after very little training. This facilitates that the LVIDdN could become a common practice in the assessment of volaemia and can guide emergency clinicians when deciding the aetiology of shock.

The validation of the SV assessment obtained from transthoracic echocardiography against thermodilution, as a way of predicting fluid-responsiveness in veterinary patients will allow the setting of a goal for fluid therapy when designing new studies. In the current situation, with the absence of a validated method to define a positive fluid response, each study states a different goal, and that prevents comparison of results. Also, setting a standard method to assess fluid-responsiveness would be of importance. As a passive leg raising test cannot be easily performed in dogs, and dogs suspected of hypovolaemia will rarely be put under mechanical ventilation, a fluid challenge will probably be the most suitable option for this purpose, testing the echocardiographic and CVC measurements against the gold-standard, thermodilution.

5. Conclusions

The EDVI was able to discriminate between hypovolaemic and euvolaemic dogs. The LVIDdN was smaller in dogs in hypovolaemic shock. The increase in CVCmax/Ao after fluid therapy suggested positive fluid response. The presence of a subjectively collapsed lumen of the LV and CVC were diagnostic of hypovolaemia and predictive of positive fluid response, respectively. A dilated CVC and right heart chambers can be predictive of increased pressure in the right atrium. Thus, a simplified echocardiographic protocol, that can be performed by non-cardiologist veterinarians in a limited time, can provide useful information about the volume status of the conscious, spontaneously breathing, clinically ill dog.

Appendix

Appendix1: Raw data of the control cohort

case number	breed	sex	age (years)	weight (kg)	SBP (mmHg)	CRT (s)	pulse	MM	HR (bpm)	LAmajor/Ao	EDVI	LVIDdN	SV (ml)	CVCmax (mm)	CVC/Ao	CVC CI
1	CS	F	3	9	120	1	0	1	80	1.9	52.3	1.5	23.1	6.0	0.42	50%
2	Mx	F	11	21	200	2	0	1	100	2.2	47.4	1.2	39.2	11.6	0.69	37%
3	ST	F	12	14	156	2	0	1	100	2.0	89.8	1.6	32.0	6.5	0.36	42%
4	Mx	M	11	31	120	2	0	1	120	2.5	83.8	1.7	56.7	9.3	0.49	45%
5	Lb	F	6	32	130	2	0	1	120	2.0	76.0	1.7	55.8	10.0	0.48	44%
6	Stz	M	10	7	130	2	0	1	100	2.4	67.6	1.7	20.0	9.0	0.82	19%
7	YT	F	13	4	140	2	0	1	140	1.9	28.5	1.6	7.3	4.8	0.55	31%
8	Pt	F	8	23	180	2	0	1	124	1.8	77.1	1.6	40.3	8.7	0.44	46%
9	Lb	M	8	35	140	2	0	1	144	1.8	58.7	1.7	66.8	13.0	0.62	25%
10	CKCS	F	9	8	150	2	0	1	110	1.7	26.8	1.6	19.8	6.5	0.46	28%
11	JRT	M	12	8	120	2	0	1	105	2.6	48.8	1.5	16.5	8.4	0.68	38%
12	BC	M	1	17	230	1	0	1	104	1.8	45.5	1.4	31.9	11.8	0.75	33%
13	Pd	M	10	12	140	2	0	1	140	2.1	46.2	1.3	25.1	8.8	0.65	50%
14	Mx	F	5	7	136	2	0	1	104	2.3	44.6	1.6	17.8	5.6	0.49	34%
15	B	M	12	17	160	2	0	1	126	2.3	44.8	1.2	19.8	13.3	0.89	38%
16	TT	M	11	15	158	2	0	1	138	2.2	40.3	1.5	15.8	7.2	0.51	64%
17	Lb	M	9	31	185	2	0	1	116	1.9	79.0	1.6	26.9	13.6	0.72	40%
18	Ch	F	8	7	120	2	0	1	120	2.3	23.0	1.3	3.9	6.0	0.62	55%
19	Lb-oo	M	9	9	140	2	0	1	116	2.1	54.5	1.3	16.6	9.5	0.73	40%

Abbreviations: B: Beagle, BC: Border collie, Ch: Chihuahua, CKCS: cavalier King Charles spaniel, CS: Cocker spaniel, F: female, JRT: Jack Russell terrier, Lb: Labrador, Lb-oo: labradoodle, M: male, Mx: mixed breed, Pd: Podenco, Pt: Pointer, ST: Staffordshire terrier, Stz: Shih-Tzu, TT: Tibetan terrier, YT: Yorkshire terrier

Appendix 2: Raw data of the study cohort.

case N	breed	sex	age (years)	weight (kg)	history	LAC 1 mmol/L	LAC 2 mmol/L	TP 1 (g/L)	TP 2 (g/L)	PCV 1 (%)	PCV 2 (%)	SBP 1 mmHg	SBP 2 mmHg	CRT 1 s	CRT 2 s	pulse 1	pulse 2	MM 1	MM 2	HR 1 bpm	HR 2 bpm	Diagnosis
1	GD	F	4	50	C	0.9	0.5	64	67	36	34	120	100	2	2	2	1	2	1	180	136	HypoAC
2	BC	M	6	21	C & RTA	1.9	2.4	62	60	38		50	120	3	2	2	0	2	1	40	120	HS/NS
3	CS	M	13	18	C	11.0	4.0	48	46	23	15	50	50	5	2	3	0	3	2	200	160	SR
4	Pk	F	8	4	C	3.3	5.6	92	82	60				2	2	1	0	1	1	80	80	Iatro HS, PHT
5	Do	M	3	37	L & RTA	1.2	2.1	62	62	50	50	118	119	2	1	0	0	0	1	126	88	PT
6	CT	M	10	9	C	5.0	2.4	40	38	26	15			4	2	2	1	2	2			SR
7	CKCS	F	14	9	V & D	1.6	1.2	50	50	32				2	2	1	0	1	1	140	110	Iatro HypoAC
8	Lu	F	11	17	A	1.8	2.6	48	48	59	51	120	160	2	2	0	0	1	1	160	136	ML
9	CKCS	M	9	8	C, V	4.0	2.0	72	68	45	44	110	116	3	2	0	0	1	1	220	160	GT
10	T	M	1	5	V & D	1.6	1.6	60	60	44		128	138	2	2	0	0	1	1	160	120	GE
11	Sts	M	11	7	V &L	2.3	1.9	64	58	48	40	140	140	3	2	2	0	2	1	160	116	IBD
12	WHWT	M	5	12	V & L	1.7	1.4	78	70	51	44	150	150	2	2	1	0	1	1	140	84	P
13	Bx	M	7	30	V & L	1.5	1.9	82	78	55	51	130	136	2	2	1	0	1	1	130	110	OFB
14	CS	M	2	13	D	2.5	1.2	70	66	55	44	136	140	4	2	2	0	2	1	160	100	Pv
15	LA	M	14	11	V & D	1.3	1.6	64	68	48	48	184	146	2	2	0	0	1	1	136	104	HAC, PHT
16	SS	F	9	15	V & D	1.6	1.7	60	58	31	25	220	240	3	2	1	0	2	2	116	100	CKD, SHT
17	Cp	M	4	6	A & D	1.5	0.8	64	60	62	52	130	130	2	2	0	0	0	1	144	88	GE
18	ST	F	9	29	V & L	3.6	3.8	64	58	65	58	168	130	1	2	1	0	0	1	166	118	OIT

1 Represents the value pre-treatment. 2 represents the value post-treatment.

Abbreviations: A: anorexia, BC: Border collie, Bx: Boxer, C: collapse, Ch: Chihuahua, CKCS: cavalier King Charles spaniel, CKD: chronic kidney disease, Cp, cockapoo, CS: Cocker spaniel, CT, Cairn terrier, D: diarrhoea, Do: Doberman, F: female, GD: great Dane, GE: gastroenteritis, GT: gastric tumour, HAC: hyperadrenocorticism, HypoAC: hypoadrenocorticism, HS: hypovolaemic shock, IBD: inflammatory bowel disease, JRT: Jack Russell terrier, L:lethargy, Lu: lurcher, LA: Lhasa Apso, Lb: Labrador, M:male, ML: multicentric lymphoma, OFB: obstructive foreign body, OIT: obstructive intestinal torsion, P: pancreatitis, Pk: Pekinese, PHT: pulmonary hypertension, PT: polytrauma, Pv: Parvovirus enteritis, RTA: road traffic accident, SHT: systemic hypertension, SR: splenic rupture, SS: springer spaniel, ST: Staffordshire terrier, Sts: Shetland sheepdog, T: Teckel, V: vomiting, WHWT: West Highland white terrier.

Appendix 3: Raw data of the study cohort (continued).

case N	LAmajor/Ao 1	LAmajor/Ao 2	EDVI 1	EDVI 2	LVIDdN 1	LVIDdN 2	CVCmax 1 (mm)	CVCmax 2 (mm)	CVCmax/Ao 1	CVCmax/Ao 2	CVC CI 1 (%)	CVC CI 2 (%)	SV 1 (ml)	SV 2 (ml)	exam time 1 (min)	exam time 2 (min)
1	1.7	1.8	50.4	81.3	1.2	1.4	14.0	16.3	0.54	0.63	53%	37%	49.9	61.3	12	9
2	2.1	2.3	60.3	80.0	1.4	1.6	12.7	12.9	0.73	0.75	23%	23%			4	4
3	1.6	2.1	20.9	54.0	0.9	1.5									3	3
4	1.6	1.7	6.7	10.4	0.6	1.1	8.1	9.8	0.82	0.98	12%	16%	3.1	6.3	5	3
5	2.0	2.0	62.3	63.8	1.3	1.3	20.5	21.2	0.92	0.92	21%	18%	76.1	118.8	4	3
6	2.0	2.3	12.3	16.0	0.6	1.1									2	3
7	2.0	2.5	52.0	82.0	1.7	1.8	9.2	9.4	0.65	0.67	57%	33%	10.8	13.7	3	3
8	1.9	1.7	58.8	62.7	1.5	1.7	10.5	12.4	0.54	0.59	65%	56%	36.2	42.6	4	3
9	3.6	3.7	87.5	90.0	2.0	2.1	7.1	8.6	0.55	0.66	20%	23%	14.8	16.2	4	3
10	1.5	1.7	32.7	24.3	1.3	1.2	8.3	8.5	0.71	0.73	47%	45%	9.1	7.9	5	3
11	2.0	2.0	22.7	35.1	1.4	1.5	8.1	8.5	0.66	0.72	23%	29%	11.4	9.2	4	2
12	2.3	2.4	26.4	40.2	1.2	1.4	7.2	9.5	0.70	0.89	35%	42%	10.4	12.1	4	2
13	1.8	2.0	36.0	63.6	1.6	1.6	15.4	15.9	0.84	0.86	32%	30%	29.8	35.4	5	2
14	1.9	2.3	50.0	63.5	1.3	1.5	6.5	12.5	0.43	0.82	31%	27%	24.2	27.8	3	2
15	2.6	2.6	35.4	32.6	1.6	1.6	10.8	11.2	0.95	1.00	32%	34%	10.4	13.3	3	5
16	1.9	2.0	43.2	69.0	1.7	1.8	10.2	10.5	0.59	0.60	31%	33%	30.0	29.6	3	2
17	1.7	1.8	11.2	18.8	0.9	1.3	6.8	7.7	0.58	0.64	34%	39%	6.1	10.5	2	2
18	1.7	1.9	45.5	46.5	1.5	1.5	9.7	13.5	0.50	0.70	48%	27%	26.3	41.4	3	4

1 Represents the value pre-treatment. 2 represents the value post-treatment.

List of references

Abbott, J. A. and Maclean, H. N. (2003) ‘Comparison of Doppler derived peak aortic velocities obtained from subcostal and apical transducer sites in healthy dogs’, *Veterinary Radiology and UltrasoundUltrasound*, 44(6), pp. 695–698. doi: 10.1111/j.1740-8261.2003.tb00533.x.

Acierno, M. J., Brown, S., Coleman, A. E., Jepson, R. E., Papich, M., Stepien, R. L., Syme, H. M., Brown, S., Coleman, A. E., Jepson, R. E., Papich, M., Stepien, R. L. and Syme, H. M. (2018) ‘ACVIM consensus statement: Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats’, *Journal of Veterinary Internal Medicine*, 32(6), pp. 1803–1822. doi: 10.1111/jvim.15331.

Ahuja, C. S., Cadotte, D. W. and Fehlings, M. (2018) ‘Spinal Cord Injury’, in *Principles of Neurological Surgery*. Elsevier Inc., pp. 518-531.e3. doi: 10.1016/B978-0-323-43140-8.00033-0.

Airapetian, N., Maizel, J., Alyamani, O., Mahjoub, Y., Lorne, E., Levrard, M., Ammenouche, N., Seydi, A., Tinturier, F., Lobjoie, E., Dupont, H. and Slama, M. (2015) ‘Does inferior vena cava respiratory variability predict fluid responsiveness in spontaneously breathing patients?’, *Critical Care*, 19(1). doi: 10.1186/s13054-015-1100-9.

Ansari, B. M., Zochios, V., Falter, F. and Klein, A. A. (2016) ‘Physiological controversies and methods used to determine fluid responsiveness: A qualitative systematic review’, *Anaesthesia*, 71(1), pp. 94–105. doi: 10.1111/anae.13246.

Aoki, T., Sunahara, H., Sugimoto, K., Ito, T., Kanai, E., Neo, S., Fujii, Y. and Wakao, Y. (2015) ‘Dynamic left ventricular outflow tract obstruction secondary to hypovolemia in a German Shepard dog with splenic hemangiosarcoma.’, *The Journal of veterinary medical science*, 77(9), pp. 1187–90. doi: 10.1292/jvms.14-0508.

Araos, J., Kenny, J. E. S., Rousseau-Blass, F. and Pang, D. S. (2020) ‘Dynamic prediction of fluid responsiveness during positive pressure ventilation: a review of the physiology underlying heart–lung interactions and a critical interpretation’, *Veterinary Anaesthesia and Analgesia*, 47(1), pp. 3–14. doi: 10.1016/j.vaa.2019.08.004.

Beal, M. W. and Hughes, D. (2000) ‘Vascular Access: Theory and Techniques in the Small Animal Emergency Patient’, *Clinical Techniques in Small Animal Practice*, 15(2), pp. 101–109. doi: 10.1053/svms.2000.6802.

Bektas, R. N., Kutter, A. P., Hartnack, S., Jud, R. S., Schnyder, M., Matos, J. M. and Bettschart-Wolfensberger, R. (2012) 'Evaluation of a minimally invasive non-calibrated pulse contour cardiac output monitor (FloTrac/Vigileo) in anaesthetized dogs', *Veterinary Anaesthesia and Analgesia*, 39(5), pp. 464–471. doi: 10.1111/j.1467-2995.2012.00741.x.

Beraud, A. S., Rizk, N. W., Pearl, R. G., Liang, D. H. and Patterson, A. J. (2013) 'Focused transthoracic echocardiography during critical care medicine training: Curriculum implementation and evaluation of proficiency', *Critical Care Medicine*, 41(8), pp. e179-81. doi: 10.1097/CCM.0b013e31828e9240.

Berkenstadt H, Friedman Z, Preisman S, Keidan I, Livingstone D, Perel A (2005) 'Pulse pressure and stroke volume variations during severe haemorrhage in ventilated dogs', *British journal of anaesthesia*, 94(6), pp. 721–726.

Bernier-Jean, A., Albert, M., Shiloh, A. L., Eisen, L. A., Williamson, D. and Beaulieu, Y. (2017) 'The Diagnostic and Therapeutic Impact of Point-of-Care Ultrasonography in the Intensive Care Unit', *Journal of Intensive Care Medicine*, 32(2), pp. 197–203. doi: 10.1177/0885066615606682.

Bighamian, R. and Hahn, J.-O. (2014) *Relationship between Stroke Volume and Pulse Pressure during Blood Volume Perturbation: A Mathematical Analysis*, *BioMed Research International*. doi: 10.1155/2014/459269.

Boag, A. K. and Hughes, D. (2005) 'Assessment and Treatment of Perfusion Abnormalities in the Emergency Patient', *Veterinary Clinics of North America Small Animal Practice*, 35, pp. 31–342. doi: 10.1016/j.cvsm.2004.10.010.

Bodson, L. and Vieillard-Baron, A. (2012) 'Respiratory variation in inferior vena cava diameter: surrogate of central venous pressure or parameter of fluid responsiveness? Let the physiology reply', *Critical Care*, 16(6) p. 181. doi: 10.1186/cc11824.

Bonagura, J. D. (1983) 'M-mode echocardiography. Basic principles.', *The Veterinary clinics of North America. Small animal practice*, 13(2), pp. 299–319. doi: 10.1016/S0195-5616(83)50031-4.

Bosiack, A. P., Mann, F. A., Dodam, J. R., Wagner-Mann, C. C. and Branson, K. R. (2010) 'Comparison of ultrasonic Doppler flow monitor, oscillometric, and direct arterial blood pressure measurements in ill dogs', *Journal of Veterinary Emergency and Critical Care*, 20(2), pp. 207–215. doi: 10.1111/j.1476-4431.2010.00520.x.

Boyd, J. H., Forbes, J., Nakada, T. A., Walley, K. R. and Russell, J. A. (2011) 'Fluid resuscitation in septic shock: A positive fluid balance and elevated central venous pressure are associated with increased mortality', *Critical Care Medicine*, 39(2), pp. 259–265. doi: 10.1097/CCM.0b013e3181feeb15.

Boyd, J. H., Sirounis, D., Maizel, J. and Slama, M. (2016) 'Echocardiography as a guide for fluid management', *Critical Care*, 20(1), p. 274. doi: 10.1186/s13054-016-1407-1.

Boysen, S. R. and Lisciandro, G. R. (2013) 'The Use of Ultrasound for Dogs and Cats in the Emergency Room AFAST and TFAST Introduction to focused assessment with sonography for trauma', *Veterinary Clinics of North America - Small Animal Practice*, 43(4), pp. 773–797. doi: 10.1016/j.cvsm.2013.03.011.

Bruegger, D., Kemming, G. I., Jacob, M., Meisner, F. G., Wojtczyk, C. J., Packert, K. B., Keipert, P. E., Simon, N. S., Habler, O. P., Becker, B. F. and Rehm, M. (2007) 'Causes of metabolic acidosis in canine hemorrhagic shock: Role of unmeasured ions', *Critical Care*, 11(6), p. R130. doi: 10.1186/cc6200.

Bucci, M., Rabozzi, R., Guglielmini, C. and Franci, P. (2017) 'Respiratory variation in aortic blood peak velocity and caudal vena cava diameter can predict fluid responsiveness in anaesthetised and mechanically ventilated dogs', *Veterinary Journal*, 227, pp. 30–35. doi: 10.1016/j.tvjl.2017.08.004.

Cambournac, M., Goy-Thollot, I., Violé, A., Boisvineau, C., Pouzot-Nevoret, C. and Barthélemy, A. (2018) 'Sonographic assessment of volaemia: Development and validation of a new method in dogs', *Journal of Small Animal Practice*, 59(3), pp. 174–182. doi: 10.1111/jsap.12759.

Campbell, F. E. and Kittleson, M. D. (2007) 'The Effect of Hydration Status on the Echocardiographic Measurements of Normal Cats', *Journal of Veterinary Internal Medicine*, 21(5), pp. 1008–1015. doi: 10.1111/j.1939-1676.2007.tb03057.x.

Canfrán, S., Cediel, R., Sández, I., Caro-Vadillo, A. and Gómez de Segura, I. A. (2015) 'Evaluation of an oesophageal Doppler device for monitoring cardiac output in anaesthetised healthy normotensive dogs', *Journal of Small Animal Practice*, 56(7), pp. 450–455. doi: 10.1111/jsap.12362.

Cannesson, M., Slieker, J., Desebbe, O., Farhat, F., Bastien, O. and Lehot, J. J. (2006) 'Prediction of fluid responsiveness using respiratory variations in left ventricular stroke

area by transoesophageal echocardiographic automated border detection in mechanically ventilated patients', *Critical Care*, 10(6), p. R171. doi: 10.1186/cc5123.

Cavanagh, A. A., Sullivan, L. A. and Hansen, B. D. (2016) 'Retrospective evaluation of fluid overload and relationship to outcome in critically ill dogs', *Journal of veterinary emergency and critical care (San Antonio, Tex. : 2001)*, 26(4), pp. 578–586. doi: 10.1111/vec.12477.

Cecconi, M., De Backer, D., Antonelli, M., Beale, R., Bakker, J., Hofer, C., Jaeschke, R., Mebazaa, A., Pinsky, M. R., Teboul, J. L., Vincent, J. L. and Rhodes, A. (2014) 'Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine', *Intensive Care Medicine*, 40(12), pp. 1795–1815. doi: 10.1007/s00134-014-3525-z.

Chetboul, V., Tidholm, A., Nicolle, A., Sampedrano, C. C., Gouni, V., Pouchelon, J. L., Lefebvre, H. P. and Concordet, D. (2005) 'Effects of animal position and number of repeated measurements on selected two-dimensional and M-mode echocardiographic variables in healthy dogs', *Journal of the American Veterinary Medical Association*, 227(5), pp. 743–747. doi: 10.2460/javma.2005.227.743.

Chew, M. S. (2012) 'Haemodynamic Monitoring Using Echocardiography in the Critically Ill: A Review', *Cardiology Research and Practice*, 2012. doi: 10.1155/2012/139537.

Cooper, E. S. and Muir, W. W. (2007) 'Continuous cardiac output monitoring via arterial pressure waveform analysis following severe hemorrhagic shock in dogs', *Critical Care Medicine*, 35(7), pp. 1724–1729. doi: 10.1097/01.CCM.0000266590.25109.F2.

Corl, K. A., George, N. R., Romanoff, J., Levinson, A. T., Chheng, D. B., Merchant, R. C., Levy, M. M. and Napoli, A. M. (2017) 'Inferior vena cava collapsibility detects fluid responsiveness among spontaneously breathing critically-ill patients', *Journal of Critical Care*, 41, pp. 130–137. doi: 10.1016/j.jcrc.2017.05.008.

Cornell, C. C., Kittleson, M. D., Della Torre, P., Häggström, J., Lombard, C. W., Pedersen, H. D., Vollmar, A. and Wey, A. (2004) 'Allometric Scaling of M-Mode Cardiac Measurements in Normal Adult Dogs', *Journal of Veterinary Internal Medicine*, 18(1), pp. 311–321. doi: 10.1111/j.1939-1676.2004.tb02551.x.

Courtice, F. C. (1943) 'The blood volume of normal animals', *The Journal of Physiology*, 102(3), pp. 290–305. doi: 10.1113/jphysiol.1943.sp004035.

Critchley, L. A., Peng, Z. Y., Fok, B. S., Lee, A. and Phillips, R. A. (2005) 'Testing the reliability of a new ultrasonic cardiac output monitor, the USCOM, by using aortic flowprobes in anesthetized dogs', *Anesthesia and Analgesia*, 100(3), pp. 748–753. doi: 10.1213/01.ANE.0000144774.42408.05.

Darnis, E., Boysen, S., Merveille, A.-C., Desquilbet, L., Chalhoub, S. and Gommeren, K. (2018) 'Establishment of reference values of the caudal vena cava by fast-ultrasonography through different views in healthy dogs', *Journal of Veterinary Internal Medicine*, 32(4), pp. 1308–1318. doi: 10.1111/jvim.15136.

Darnis, E., Merveille, A. C., Desquilbet, L., Boysen, S. and Gommeren, K. (2019) 'Interobserver agreement between non-cardiologist veterinarians and a cardiologist after a 6-hour training course for echographic evaluation of basic echocardiographic parameters and caudal vena cava diameter in 15 healthy Beagles', *Journal of Veterinary Emergency and Critical Care*, 29(5), pp. 495–504. doi: 10.1111/vec.12883.

Davis, H., Jensen, T., Johnson, A., Knowles, P., Meyer, R., Rucinsky, R. and Shafford, H. (2013) 'Veterinary practice guidelines 2013 AAHA/AAFP Fluid Therapy Guidelines for Dogs and Cats', *Journal of the American Animal Hospital Association*, 49, pp. 149–159. doi: 10.5326/JAAHA-MS-5868.

Day, T. K., Boyle, C. R. and Holland, M. (2007) 'Lack of agreement between thermodilution and echocardiographic determination of cardiac output during normovolemia and acute hemorrhage in clinically healthy, anesthetized dogs', *Journal of Veterinary Emergency and Critical Care*, 17(1), pp. 22–31. doi: 10.1111/j.1476-4431.2006.00208.x.

DiBartola, S. P. (2012) *Fluid, Electrolyte, and Acid-base disorders in Small Animal Practice*. 4th edn. St. Louis: Elsevier Inc.

Dipti, A., Soucy, Z., Surana, A. and Chandra, S. (2012) 'Role of inferior vena cava diameter in assessment of volume status: A meta-analysis', *American Journal of Emergency Medicine*, 30(8). doi: 10.1016/j.ajem.2011.10.017.

Donati, P. A., Guevara, J. M., Ardiles, V., Guillemi, E. C., Londoño, L. and Dubin, A. (2020) 'Caudal vena cava collapsibility index as a tool to predict fluid responsiveness in dogs', *Journal of Veterinary Emergency and Critical Care*, 30(6), pp. 677-686 doi: 10.1111/vec.13009.

Drozdzynska, M. J., Chang, Y. M., Stanzani, G. and Pelligand, L. (2018) ‘Evaluation of the dynamic predictors of fluid responsiveness in dogs receiving goal-directed fluid therapy’, *Veterinary Anaesthesia and Analgesia*, 45(1), pp. 22–30. doi: 10.1016/j.vaa.2017.06.001.

Dukes-McEwan, J., French, A. T. and Corcoran, B. M. (2002) ‘Doppler echocardiography in the dog: Measurement variability and reproducibility’, *Veterinary Radiology and Ultrasound*, 43(2), pp. 144–152. doi: 10.1111/j.1740-8261.2002.tb01662.x.

Endo, Y., Kawase, K., Miyasho, T., Sano, T., Yamashita, K. and Muir, W. W. (2017) ‘Plethysmography variability index for prediction of fluid responsiveness during graded haemorrhage and transfusion in sevoflurane-anaesthetized mechanically ventilated dogs’, *Veterinary Anaesthesia and Analgesia*, 44(6), pp. 1303–1312. doi: 10.1016/j.vaa.2017.07.007.

Ettinger, S., Feldman, E. and Cote, E. (2016) *Textbook of Veterinary Internal Medicine. Diseases of the dog anf the cat.* 8th edn. Philadelphia: Saunders.

Feissel, M., Michard, F., Mangin, I., Ruyer, O., Faller, J. and Teboul, J. (2001) ‘Respiratory changes in aortic blood velocity as an indicator of fluid responsiveness in ventilated patients with septic shock’, *Chest*, 119, pp. 867–873.

Feissel, M., Michard, F., Faller, J. P. and Teboul, J. L. (2004) ‘The respiratory variation in inferior vena cava diameter as a guide to fluid therapy’, *Intensive Care Medicine*, 30(9), pp. 1834–1837. doi: 10.1007/s00134-004-2233-5.

Fine, D. M., Durham Jr, H. E., Rossi, N. F., Spier, A. W., Selting, K. and Rubin, L. J. (2010) ‘Echocardiographic Assessment of Hemodynamic Changes Produced by Two Methods of Inducing Fluid Deficit in Dogs’, *Journal of Veterinary Internal Medicine*, 24(2), pp. 348–353. doi: 10.1111/j.1939-1676.2009.0448.x.

Finn-Bodner, S. T. and Hudson, J. A. (1998) ‘Abdominal vascular sonography.’, *The Veterinary clinics of North America. Small animal practice*, 28(4), pp. 887–942. doi: 10.1016/s0195-5616(98)50083-6.

Finnerty, N. M., Panchal, A. R., Boulger, C., Vira, A., Bischof, J. J., Amick, C., Way, D. P. and Bahner, D. P. (2017) ‘Inferior vena cava measurement with ultrasound: What is the best view and best mode?’, *Western Journal of Emergency Medicine*. eScholarship, pp. 496–501. doi: 10.5811/westjem.2016.12.32489.

Fleming, S., Gill, P., Jones, C., Taylor, J. A., Van Den Bruel, A., Heneghan, C., Roberts, N. and Thompson, M. (2015) 'The diagnostic value of capillary refill time for detecting serious illness in children: A systematic review and meta-analysis', *PLoS ONE*, 10(9). doi: 10.1371/journal.pone.0138155.

Gentile-Solomon, J. M. and Abbott, J. A. (2016) 'Conventional echocardiographic assessment of the canine right heart: reference intervals and repeatability', *Journal of Veterinary Cardiology*, 18(3), pp. 234–247. doi: 10.1016/j.jvc.2016.05.002.

Gillespie, Í., Rosenstein, P. G. and Hughes, D. (2017) 'Update: Clinical Use of Plasma Lactate', *Veterinary Clinics of North America - Small Animal Practice*, pp. 325–342. doi: 10.1016/j.cvsm.2016.10.011.

Goucher, T. K., Hartzell, A. M., Seales, T. S., Anmuth, A. S., Zanghi, B. M. and Otto, C. M. (2019) 'Evaluation of skin turgor and capillary refill time as predictors of dehydration in exercising dogs', *American Journal of Veterinary Research*, 80(2), pp. 123–128. doi: 10.2460/ajvr.80.2.123.

Gui, J., Yang, Z., Ou, B., Xu, A., Yang, F., Chen, Q., Jiang, L. and Tang, W. (2018) 'Is the Collapsibility Index of the Inferior Vena Cava an Accurate Predictor for the Early Detection of Intravascular Volume Change?', *SHOCK*, 49(1), pp. 29–32. doi: 10.1097/SHK.0000000000000932.

Gunkel, C. I., Valverde, A., Morey, T. E., Hernandez, J. and Robertson, S. A. (2004) 'Comparison of non-invasive cardiac output measurement by partial carbon dioxide rebreathing with the lithium dilution method in anesthetized dogs', *Journal of Veterinary Emergency and Critical Care*, 14(3), pp. 187–195. doi: 10.1111/j.1534-6935.2004.04017.x.

Haberman, C. E., Kang, C. W., Morgan, J. D. and Brown, S. A. (2006) 'Evaluation of oscillometric and Doppler ultrasonic methods of indirect blood pressure estimation in conscious dogs', *Canadian Journal of Veterinary Research*, 70(3), pp. 211–217.

Hammes, K., Novo Matos, J., Baron Toaldo, M. and Glaus, T. (2016) 'Hypovolemia induced systolic anterior motion of the mitral valve in two dogs', *Journal of Veterinary Cardiology*, 18(4), pp. 367–371. doi: 10.1016/j.jvc.2016.06.003.

Heerdt, P. M., Wagner, C. L., DeMais, M. and Savarese, J. J. (2011) 'Noninvasive cardiac output monitoring with bioreactance as an alternative to invasive instrumentation for

preclinical drug evaluation in beagles.', *Journal of pharmacological and toxicological methods*, 64(2), pp. 111–8. doi: 10.1016/j.vascn.2011.03.006.

Herreria-Bustillo, V. J., Fitzgerald, E. and Humm, K. R. (2019) 'Caval-aortic ratio and caudal vena cava diameter in dogs before and after blood donation', *Journal of Veterinary Emergency and Critical Care*, 29(6), pp. 643–646. doi: 10.1111/vec.12900.

Höglund, K., Hanås, S., Carnabuci, C., Ljungvall, I., Tidholm, A. and Häggström, J. (2012) 'Blood Pressure, Heart Rate, and Urinary Catecholamines in Healthy Dogs Subjected to Different Clinical Settings', *Journal of Veterinary Internal Medicine*, 26(6), pp. 1300–1308. doi: 10.1111/j.1939-1676.2012.00999.x.

Huss, B. T., Anderson, M. A., Branson, K. R., Wagner-Mann, C. C. and Mann, F. A. (1995) 'Evaluation of pulse oximeter probes and probe placement in healthy dogs.', *Journal of the American Animal Hospital Association*, 31(1), pp. 9–14. doi: 10.5326/15473317-31-1-9.

Itami, T., Endo, Y., Hanazono, K., Ishizuka, T., Tamura, J., Miyoshi, K., Sano, T. and Yamashita, K. (2016) 'Comparison of cardiac output measurements using transpulmonary thermodilution and conventional thermodilution techniques in anaesthetized dogs with fluid overload.', *Veterinary anaesthesia and analgesia*, 43(4), pp. 388–96. doi: 10.1111/vaa.12331.

Joannidis, M., Druml, W., Forni, L. G., Groeneveld, A. B. J., Honore, P., Oudemans-van Straaten, H. M., Ronco, C., Schetz, M. R. C. and Woittiez, A. J. (2010) 'Prevention of acute kidney injury and protection of renal function in the intensive care unit', *Intensive Care Medicine*, 36(3), pp. 392–411. doi: 10.1007/s00134-009-1678-y.

Johnson, P. (2016) 'Practical Assessment of Volume Status in Daily Practice', *Topics in Companion Animal Medicine*, 31(3), pp. 86–93. doi: 10.1053/j.tcam.2016.08.002.

Josephs, W. and Odenthal, H. J. (1995) 'Cardiac manifestations of terminal renal failure', *Deutsche Medizinische Wochenschrift*. Georg Thieme Verlag, pp. 141–144. doi: 10.1055/s-2008-1047804.

Keene, B. W., Atkins, C. E., Bonagura, J. D., Fox, P., Häggström, J., Fuentes, V. L., Oyama, M. A., Rush, J. E., Stepien, R. and Uechi, M. (2019) 'ACVIM consensus guidelines for the diagnosis and treatment of myxomatous mitral valve disease in dogs', *Journal of Veterinary Internal Medicine*, 33(3). doi: 10.1111/jvim.15488.

Kent, A., Bahner, D. P., Boulger, C. T., Eiferman, D. S., Adkins, E. J., Evans, D. C., Springer, A. N., Balakrishnan, J. M., Valiyaveedan, S., Galwankar, S. C., Njoku, C., Lindsey, D. E., Yeager, S., Roelant, G. J. and Stawicki, S. P. A. (2013) 'Sonographic evaluation of intravascular volume status in the surgical intensive care unit: A prospective comparison of subclavian vein and inferior vena cava collapsibility index', *Journal of Surgical Research*, 184(1), pp. 561–566. doi: 10.1016/j.jss.2013.05.040.

Kircher, B. J., Himelman, R. B. and Schiller, N. B. (1990) 'Noninvasive estimation of right atrial pressure from the inspiratory collapse of the inferior vena cava', *The American Journal of Cardiology*, 66(4), pp. 493–496. doi: 10.1016/0002-9149(90)90711-9.

Kosiak, W., Swieton, D. and Piskunowicz, M. (2008) 'Sonographic inferior vena cava/aorta diameter index, a new approach to the body fluid status assessment in children and young adults in emergency ultrasound-preliminary study', *American Journal of Emergency Medicine*, 26(3), pp. 320–325. doi: 10.1016/j.ajem.2007.07.012.

Kutter, A. P. N., Bettschart-Wolfensberger, R., Romagnoli, N. and Bektas, R. N. (2016) 'Evaluation of agreement and trending ability between transpulmonary thermodilution and calibrated pulse contour and pulse power cardiac output monitoring methods against pulmonary artery thermodilution in anesthetized dogs', *Journal of Veterinary Emergency and Critical Care*, 26(4), pp. 531–540. doi: 10.1111/vec.12439.

Kwak, J., Yoon, H., Kim, J., Kim, M. and Eom, K. (2018) 'Ultrasonographic measurement of caudal vena cava to aorta ratios for determination of volume depletion in normal beagle dogs', *Veterinary Radiology & Ultrasound*, 59(2), pp. 203–211. doi: 10.1111/vru.12566.

Leung, J. M. and Levine, E. H. (1994) 'Left ventricular end-systolic cavity obliteration as an estimate of intraoperative hypovolemia', *Anesthesiology*, 81(5), pp. 1102–1109. doi: 10.1097/00000542-199411000-00003.

Levy, B., Desebbe, O., Montemont, C. and Gibot, S. (2008) 'Increased aerobic glycolysis through β2 stimulation is a common mechanism involved in lactate formation during shock states', *Shock*, 30(4), pp. 417–421. doi: 10.1097/SHK.0b013e318167378f.

Lisciandro, G. R. (2011) 'Abdominal and thoracic focused assessment with sonography for trauma, triage, and monitoring in small animals', *Journal of Veterinary Emergency and Critical Care*,21(2) pp. 104–122. doi: 10.1111/j.1476-4431.2011.00626.x.

Long, E., Oakley, E., Duke, T. and Babl, F. E. (2017) 'Does Respiratory Variation in

Inferior Vena Cava Diameter Predict Fluid Responsiveness: A Systematic Review and Meta-Analysis', *Shock*, pp. 550–559. doi: 10.1097/SHK.0000000000000801.

Lopes, P. C. F., Sousa, M. G., Camacho, A. A., Carareto, R., Nishimori, C. T. D., Santos, P. S. P. and Nunes, N. (2010) 'Comparison between two methods for cardiac output measurement in propofol-anesthetized dogs: Thermodilution and Doppler', *Veterinary Anaesthesia and Analgesia*, 37(5), pp. 401–408. doi: 10.1111/j.1467-2995.2010.00552.x.

Mandelbaum, A. and Ritz, E. (1996) 'Vena cava diameter measurement for estimation of dry weight in haemodialysis patients', in *Nephrology Dialysis Transplantation*. Oxford University Press, pp. 24–27. doi: 10.1093/ndt/11.supp2.24.

Mantovani, M. M., Fantoni, D. T., Gimenes, A. M., de Castro, J. R., Flor, P. B., Ida, K. K. and Schwartz, D. S. (2017) 'Clinical monitoring of cardiac output assessed by transoesophageal echocardiography in anaesthetised dogs: A comparison with the thermodilution technique', *BMC Veterinary Research*, 13(1), p. 325. doi: 10.1186/s12917-017-1227-9.

Marik, P. E., Cavallazzi, R., Vasu, T. and Hirani, A. (2009) 'Dynamic changes in arterial waveform derived variables and fluid responsiveness in mechanically ventilated patients: A systematic review of the literature', *Critical Care Medicine*, 37(9),pp. 2642–2647. doi: 10.1097/CCM.0b013e3181a590da.

Marik, P. E. and Cavallazzi, R. (2013) 'Does the central venous pressure predict fluid responsiveness? An updated meta-analysis and a plea for some common sense.', *Critical care medicine*, 41(7), pp. 1774–81. doi: 10.1097/CCM.0b013e31828a25fd.

Marik, P. E. and Lemson, J. (2014) 'Fluid responsiveness: An evolution of our understanding', *British Journal of Anaesthesia*. Oxford University Press, pp. 617–620. doi: 10.1093/bja/aet590.

Marik, P. E., Monnet, X. and Teboul, J. L. (2011) 'Hemodynamic parameters to guide fluid therapy', *Annals of Intensive Care*. Springer Verlag. doi: 10.1186/2110-5820-1-1.

Marshall, K., Thomovsky, E., Johnson, P. and Brooks, A. (2016) 'A Review of Available Techniques for Cardiac Output Monitoring', *Topics in Companion Animal Medicine*, 31(3), pp. 100–108. doi: 10.1053/j.tcam.2016.08.006.

Marshall, K., Thomovsky, E., Brooks, A., Johnson, P., Kin Lim, C. and Gan Heng, H. (2018) 'Ultrasound measurements of the caudal vena cava before and after blood donation

in 9 greyhound dogs', *Canadian Veterinary Journal*, 59(9), pp. 973–980.

Martin, L. D., Ziegelstein, R. C., Howell, E. E., Martire, C., Hellmann, D. B. and Hirsch, G. A. (2013) 'Hospitalists' ability to use hand-carried ultrasound for central venous pressure estimation after a brief training intervention: A pilot study', *Journal of Hospital Medicine*, 8(12), pp. 711–714. doi: 10.1002/jhm.2103.

McGowan, J. H. and Cleland, J. G. F. (2003) 'Reliability of reporting left ventricular systolic function by echocardiography: A systematic review of 3 methods', *American Heart Journal*, 146(3), pp. 388–397. doi: 10.1016/S0002-8703(03)00248-5.

McGrotty, Y. and Knottenbelt, C. (2002) 'Significance of plasma protein abnormalities in dogs and cats', *In Practice*, 24(9), pp. 512–517. doi: 10.1136/inpract.24.9.512.

McLean, A. S. (2016) 'Echocardiography in shock management', *Critical Care*, 20(1), p. 275. doi: 10.1186/s13054-016-1401-7.

Meneghini, C., Rabozzi, R. and Franci, P. (2016) 'Correlation of the ratio of caudal vena cava diameter and aorta diameter with systolic pressure variation in anesthetized dogs', *American Journal of Veterinary Research*, 77(2), pp. 137–143. doi: 10.2460/ajvr.77.2.137.

Mercat, A., Diehl, J.-L., Meyer, G., Teboul, J.-L. and Sors, H. (1999) 'Hemodynamic effects of fluid loading in acute massive pulmonary embolism', *Critical Care Medicine*, 27(3), pp. 540–544. doi: 10.1097/00003246-199903000-00032.

Miller, A., Fficm, M. F., Mandeville, J., Bs, M. B., Mrcp, B. and Fficm, F. (2016) 'Predicting and measuring fluid responsiveness with echocardiography'. *Echo research and practice*,3(2),G1-G12. doi: 10.1530/ERP-16-0008.

Millington, S. J. (2019) 'Ultrasound assessment of the inferior vena cava for fluid responsiveness: easy, fun, but unlikely to be helpful', *Canadian Journal of Anesthesia*, 66(6), pp. 633–638. doi: 10.1007/s12630-019-01357-0.

Monnet, X., Marik, P. E. and Teboul, J. L. (2016a) 'Prediction of fluid responsiveness: an update', *Annals of Intensive Care*, 6(1). doi: 10.1186/s13613-016-0216-7.

Monnet, X., Marik, P. and Teboul, J. L. (2016b) 'Passive leg raising for predicting fluid responsiveness: a systematic review and meta-analysis', *Intensive Care Medicine*, 42(12), pp. 1935–1947. doi: 10.1007/s00134-015-4134-1.

Mookadam, F., Warsame, T. A., Yang, H. S., Emani, U. R., Appleton, C. P. and Raslan, S.

F. (2011) ‘Effect of positional changes on inferior vena cava size’, *European Journal of Echocardiography*, 12(4), pp. 322–325. doi: 10.1093/EJECHOCARD/JER018.

Morgaz, J., Granados, M. del M., Muñoz-Rascón, P., Dominguez, J. M., Fernández-Sarmiento, J. A., Gómez-Villamandos, R. J. and Navarrete, R. (2014) ‘Comparison of thermodilution, lithium dilution, and pulse contour analysis for the measurement of cardiac output in 3 different hemodynamic states in dogs’, *Journal of Veterinary Emergency and Critical Care*, 24(5), pp. 562–570. doi: 10.1111/vec.12219.

Moulinier, L., Venet, T., Schiller, N. B., Kurtz, T. W., Curtis Morris, R. and Sebastian, A. (1991) ‘Measurement of aortic blood flow by doppler echocardiography: Day to day variability in normal subjects and applicability in clinical research’, *Journal of the American College of Cardiology*, 17(6), pp. 1326–1333. doi: 10.1016/S0735-1097(10)80143-3.

Muir, W. W., Kijtawornrat, A., Ueyama, Y., Radecki, S. V. and Hamlin, R. L. (2011) ‘Effects of intravenous administration of lactated ringer’s solution on hematologic, serum biochemical, rheological, hemodynamic, and renal measurements in healthy isoflurane-anesthetized dogs’, *Journal of the American Veterinary Medical Association*, 239(5), pp. 630–637. doi: 10.2460/javma.239.5.630.

Muir, W. W., Ueyama, Y., Pedraza-Toscano, A., Vargas-Pinto, P., Delrio, C. L., George, R. S., Youngbloods, B. L. and Hamlin, R. L. (2014) ‘Arterial blood pressure as a predictor of the response to fluid administration in euvolemic nonhypotensive or hypotensive isoflurane-anesthetized dogs’, *Journal of the American Veterinary Medical Association*, 245(9), pp. 1021–1027. doi: 10.2460/javma.245.9.1021.

Van der Mullen, J., Wise, R., Vermeulen, G., Moonen, P.-J. and Malbrain, M. L. N. G. (2018) ‘Assessment of hypovolaemia in the critically ill’, *Anestezjologia Intensywna Terapia*, 50(2), pp. 141–149. doi: 10.5603/AIT.a2017.0077.

Muller, L., Toumi, M., Bousquet, P. J., Riu-Poulenc, B., Louart, G., Candela, D., Zoric, L., Suehs, C., De La Coussaye, J. E., Molinari, N. and Lefrant, J. Y. (2011) ‘An increase in aortic blood flow after an infusion of 100 ml colloid over 1 minute can predict fluid responsiveness: The mini-fluid challenge study’, *Anesthesiology*, 115(3), pp. 541–547. doi: 10.1097/ALN.0b013e318229a500.

Oakley, R. E., Olivier, B., Eyster, G. E. and Hauptman, J. G. (1997) ‘Experimental Evaluation of Central Venous Pressure Monitoring in the Dog’, *Journal of the American*

Animal Hospital Association, 33(1), pp. 77–82. doi: 10.5326/15473317-33-1-77.

Orso, D., Paoli, I., Piani, T., Cilenti, F. L., Cristiani, L. and Guglielmo, N. (2020) 'Accuracy of Ultrasonographic Measurements of Inferior Vena Cava to Determine Fluid Responsiveness: A Systematic Review and Meta-Analysis', *Journal of Intensive Care Medicine*, 35(4), pp. 354–363. doi: 10.1177/0885066617752308.

Pachtinger, G. E. and Drobatz, K. (2008) 'Assessment and Treatment of Hypovolemic States', *Veterinary Clinics of North America: Small Animal Practice*, 38(3), pp. 629–643. doi: 10.1016/j.cvsm.2008.01.009.

Pang, D. S. and Boysen, S. (2007) 'Lactate in veterinary critical care: Pathophysiology and management', *Journal of the American Animal Hospital Association*, 43(5), pp. 270–279. doi: 10.5326/0430270.

Perel, A., Pizov, R. and Cotev, S. (1987) 'The systolic pressure variation is a sensitive indicator of hypovolemia in ventilated dogs subjected to graded hemorrhage', *Anesthesiology*, 67, pp. 498–502.

Phillips, R. A., Smith, B. E. and Madigan, V. M. (2017) 'Stroke Volume Monitoring: Novel Continuous Wave Doppler Parameters, Algorithms and Advanced Noninvasive Haemodynamic Concepts', *Current Anesthesiology Reports*, 7(4), pp. 387–398. doi: 10.1007/s40140-017-0235-4.

Porter, A. E., Rozanski, E. A., Sharp, C. R., Dixon, K. L., Price, L. L. and Shaw, S. P. (2013) 'Evaluation of the shock index in dogs presenting as emergencies.', *Journal of veterinary emergency and critical care (San Antonio, Tex. : 2001)*, 23(5), pp. 538–44. doi: 10.1111/vec.12076.

Porter, T. R., Shillcutt, S. K., Adams, M. S., Desjardins, G., Glas, K. E., Olson, J. J. and Troughton, R. W. (2015) 'Guidelines for the use of echocardiography as a monitor for therapeutic intervention in adults: A report from the american society of echocardiography', *Journal of the American Society of Echocardiography*, 28(1), pp. 40–56. doi: 10.1016/j.echo.2014.09.009.

Pouchelon, J. L., Atkins, C. E., Bussadori, C., Oyama, M. A., Vaden, S. L., Bonagura, J. D., Chetboul, V., Cowgill, L. D., Elliot, J., Francey, T., Grauer, G. F., Luis Fuentes, V., Sydney Moise, N., Polzin, D. J., Van Dongen, A. M. and Van Israël, N. (2015) 'Cardiovascular-renal axis disorders in the domestic dog and cat: A veterinary consensus

statement', *Journal of Small Animal Practice*, 56(9), pp. 537–552. doi: 10.1111/jsap.12387.

Rabozzi, R., Oricco, S., Meneghini, C., Bucci, M. and Franci, P. (2020) 'Evaluation of the caudal vena cava diameter to abdominal aortic diameter ratio and the caudal vena cava respiratory collapsibility for predicting fluid responsiveness in a heterogeneous population of hospitalized conscious dogs', *Journal of Veterinary Medical Science*, 82(3), pp. 337–344. doi: 10.1292/jvms.19-0028.

Rabozzi, R. and Franci, P. (2014) 'Use of systolic pressure variation to predict the cardiovascular response to mini-fluid challenge in anaesthetised dogs', *Veterinary Journal*, 202(2), pp. 367–371. doi: 10.1016/j.tvjl.2014.08.022.

Rady, M. Y., Rivers, E. P. and Nowak, R. M. (1996) 'Resuscitation of the critically ill in the ED: Responses of blood pressure, heart rate, shock index, central venous oxygen saturation, and lactate', *American Journal of Emergency Medicine*, pp. 218–225. doi: 10.1016/S0735-6757(96)90136-9.

Reece, W. O., Erickson, H. H., Goff, J. P. and Uemura, E. E. (2015) *Dukes' physiology of domestic animals. – 13th edn*. Edited by H. H. Erickson, J. P. Goff, and E. E. Uemura. Wiley.

Reminga, C. L., Silverstein, D. C., Drobatz, K. J. and Clarke, D. L. (2018) 'Evaluation of the placement and maintenance of central venous jugular catheters in critically ill dogs and cats.', *Journal of veterinary emergency and critical care (San Antonio, Tex. : 2001)*, 28(3), pp. 232–243. doi: 10.1111/vec.12714.

Robinson, D. A., Kiefer, K., Bassett, R. and Quandt, J. (2016) 'Autotransfusion in dogs using a 2-syringe technique', *Journal of Veterinary Emergency and Critical Care*, 26(6), pp. 766–774. doi: 10.1111/vec.12476.

Rosenberg, A. L., Dechert, R. E., Park, P. K. and Bartlett, R. H. (2009) 'Review of a large clinical series: Association of cumulative fluid balance on outcome in acute lung injury: A retrospective review of the ARDSnet tidal volume study cohort', *Journal of Intensive Care Medicine*, 24(1), pp. 35–46. doi: 10.1177/0885066608329850.

Rosenstein, P. G., Tennent-Brown, B. S. and Hughes, D. (2018) 'Clinical use of plasma lactate concentration. Part 2: Prognostic and diagnostic utility and the clinical management of hyperlactatemia', *Journal of Veterinary Emergency and Critical Care*, 28(2), pp. 106–

121. doi: 10.1111/vec.12706.

Salahuddin, N., Sammani, M., Hamdan, A., Joseph, M., Al-Nemary, Y., Alquaiz, R., Dahli, R. and Maghrabi, K. (2017) 'Fluid overload is an independent risk factor for acute kidney injury in critically Ill patients: results of a cohort study', *BMC Nephrology*, 18(1), pp. 1–8. doi: 10.1186/s12882-017-0460-6.

Sano, H., Seo, J., Wightman, P., Cave, N. J., Gieseg, M. A., Johnson, C. B. and Chambers, P. (2018) 'Evaluation of pulse pressure variation and pleth variability index to predict fluid responsiveness in mechanically ventilated isoflurane-anesthetized dogs', *Journal of Veterinary Emergency and Critical Care*, 28(4), pp. 301–309. doi: 10.1111/vec.12728.

Sasaki, K., Mutoh, Tatsushi, Mutoh, Tomoko, Kawashima, R. and Tsubone, H. (2017) 'Electrical velocimetry for noninvasive cardiac output and stroke volume variation measurements in dogs undergoing cardiovascular surgery', *Veterinary Anaesthesia and Analgesia*, 44(1), pp. 7–16. doi: 10.1111/vaa.12380.

Sasaki, K., Mutoh, T., Yamamoto, S., Taki, Y. and Kawashima, R. (2018) 'Comparison of noninvasive dynamic indices of fluid responsiveness among different ventilation modes in dogs recovering from experimental cardiac surgery', *Medical Science Monitor*, 24, pp. 7736–7741. doi: 10.12659/MSM.910135.

Scansen, B. A., Bonagura, J. D., Schober, K. E. and Muir, W. W. (2009) 'Evaluation of a commercial ultrasonographic hemodynamic recording system for the measurement of cardiac output in dogs', *American Journal of Veterinary Research*, 70(7), pp. 862–868. doi: 10.2460/ajvr.70.7.862.

Sharkey, L. C. and Wellman, M. L. (2013) 'Use of Lactate in Small Animal Clinical Practice', *Veterinary Clinics of North America - Small Animal Practice*, 43 (6), pp. 1287–1297. doi: 10.1016/j.cvsm.2013.07.012.

Shih, A., Maisenbacher, H. W., Bandt, C., Ricco, C., Bailey, J., Rivera, J. and Estrada, A. (2011) 'Assessment of cardiac output measurement in dogs by transpulmonary pulse contour analysis', *Journal of Veterinary Emergency and Critical Care*, 21(4), pp. 321–327. doi: 10.1111/j.1476-4431.2011.00651.x.

Shore, R., Holt, J. P. and Knoefel, P. K. (1945) 'Determination of cardiac output in the dog by the Fick procedure', *American Journal of Physiology-Legacy Content*, 143(5), pp. 709–714. doi: 10.1152/ajplegacy.1945.143.5.709.

Silverstein, D. C., Aldrich, J., Haskins, S. C., Drobatz, K. J. and Cowgill, L. D. (2005) 'Assessment of changes in blood volume in response to resuscitative fluid administration in dogs', *Journal of Veterinary Emergency and Critical Care*, 15(3), pp. 185–192. doi: 10.1111/j.1476-4431.2005.00138.x.

Silverstein, D. C. and Hopper, K. (2014) *Small animal critical care medicine*. 2nd edn. New Jersey: Elsevier Health Sciences. doi: 10.1016/B978-1-4557-0306-7.00216-6.

Stawicki, S. P., Braslow, B. M., Panebianco, N. L., Kirkpatrick, J. N., Gracias, V. H., Hayden, G. E. and Dean, A. J. (2009) 'Intensivist Use of Hand-Carried Ultrasonography to Measure IVC Collapsibility in Estimating Intravascular Volume Status: Correlations with CVP', *Journal of the American College of Surgeons*, 209(1), pp. 55–61. doi: 10.1016/j.jamcollsurg.2009.02.062.

Steingart, R. M., Meller, J., Barovick, J., Patterson, R., Herman, M. V and Teichholz, L. E. (1980) 'Pulsed doppler echocardiographic measurement of beat-to-beat changes in stroke volume in dogs.', *Circulation*, 62(3), pp. 542–8. doi: 10.1161/01.cir.62.3.542.

Stevenson, C. K., Kidney, B. A., Duke, T., Snead, E. C. R., Mainar-Jaime, R. C. and Jackson, M. L. (2007) 'Serial blood lactate concentrations in systemically ill dogs', *Veterinary Clinical Pathology*, 36(3), pp. 234–239. doi: 10.1111/j.1939-165X.2007.tb00217.x.

Strohm, L. E., Visser, L. C., Chapel, E. H., Drost, W. T. and Bonagura, J. D. (2018) 'Two-dimensional, long-axis echocardiographic ratios for assessment of left atrial and ventricular size in dogs', *Journal of Veterinary Cardiology*, 20(5), pp. 330–342. doi: 10.1016/j.jvc.2018.07.008.

Sugimoto, K., Kawase, N., Aoki, T. and Fujii, Y. (2019) 'Effects of dehydration on echocardiographic diastolic parameters in healthy cats.', *Journal of veterinary science*, 20(3), p. e18. doi: 10.4142/jvs.2019.20.e18.

Taguchi, H., Ichinose, K., Tanimoto, H., Sugita, M., Tashiro, M. and Yamamoto, T. (2011) 'Stroke volume variation obtained with Vigileo/FloTrac™ system during bleeding and fluid overload in dogs', *Journal of Anesthesia*, 25(4), pp. 563–568. doi: 10.1007/s00540-011-1175-7.

Tavernier, B., Makhotine, O., Lebuffe, G., Dupont, J. and Scherpereel, P. (1998) 'Systolic pressure variation as a guide to fluid therapy in patients with sepsis-induced hypotension',

Anesthesiology, 89(6), pp. 1313–1321. doi: 10.1097/00000542-199812000-00007.

Thomas, W. P., Gaber, C. E., Jacobs, G. J., Kaplan, P. M., Lombard, C. W., Vet, M., Moise, N. S. and Moses, B. L. (1993) 'Recommendations for Standards in Transthoracic Two-Dimensional Echocardiography in the Dog and Cat', *Journal of Veterinary Internal Medicine*, 7(4), pp. 247–252. doi: 10.1111/j.1939-1676.1993.tb01015.x.

Tilley, L., Smith, F., Oyama, M. and Sleeper, M. (2008) *Manual of Canine and Feline Cardiology*. 4th edn. St. Louis: Elsevier. doi: 10.1016/B978-1-4160-2398-2.X0032-0.

Tuplin, M. C., Romero, A. E. and Boysen, S. R. (2017) 'Influence of the Respiratory Cycle on Caudal Vena Cava Diameter Measured by Sonography in Healthy Foals: A Pilot Study.', *Journal of veterinary internal medicine*, 31(5), pp. 1556–1562. doi: 10.1111/jvim.14793.

Uemura, K., Kawada, T., Inagaki, M. and Sugimachi, M. (2013) 'A minimally invasive monitoring system of cardiac output using aortic flow velocity and peripheral arterial pressure profile', *Anesthesia and Analgesia*, 116(5), pp. 1006–1017. doi: 10.1213/ANE.0b013e31828a75bd.

Underwood, C., Norton, J. L., Nolen-Walston, R. D., Dallap-Schaer, B. L., Boston, R. and Slack, J. (2011) 'Echocardiographic changes in heart size in hypohydrated horses.', *Journal of veterinary internal medicine*, 25(3), pp. 563–9. doi: 10.1111/j.1939-1676.2010.0612.x.

Vail, D. M., Ogilvie, G. K., Fettman, M. J. and Wheeler, S. L. (1990) 'Exacerbation of Hyperlactatemia by Infusion of Lactated Ringer's Solution in Dogs With Lymphoma', *Journal of Veterinary Internal Medicine*, 4(5), pp. 228–232. doi: 10.1111/j.1939-1676.1990.tb03113.x.

Valverde, A., Gianotti, G., Rioja, E. and Hathway, A. (2011) 'Comparison of cardiac output determined by arterial pulse pressure waveform analysis method (FloTrac/Vigileo) versus lithium dilution method in anesthetized dogs', *Journal of Veterinary Emergency and Critical Care*, 21(4), pp. 328–334. doi: 10.1111/j.1476-4431.2011.00650.x.

Valverde, A., Gianotti, G., Rioja-Garcia, E. and Hathway, A. (2012) 'Effects of high-volume, rapid-fluid therapy on cardiovascular function and hematological values during isoflurane-induced hypotension in healthy dogs', *Canadian Journal of Veterinary Research*, 76(2), pp. 99–108.

De Vecchis, R., Baldi, C., Giandomenico, G., Di Maio, M., Giasi, A. and Cioppa, C. (2016) 'Estimating Right Atrial Pressure Using Ultrasounds: An Old Issue Revisited With New Methods', *Journal of Clinical Medicine Research*, 8(8), pp. 569–574. doi: 10.14740/jocmr2617w.

Vientós-Plotts, A. I., Wiggen, K. E., Lisciandro, G. R. and Reinero, C. R. (2019) 'The utility of point-of-care ultrasound right-sided cardiac markers as a screening test for moderate to severe pulmonary hypertension in dogs', *Veterinary Journal*, 250, pp. 6–13. doi: 10.1016/j.tvjl.2019.05.013.

Vincent, J. L. and De Backer, D. (2013) 'Circulatory shock', *New England Journal of Medicine*. Massachussetts Medical Society, pp. 1726–1734. doi: 10.1056/NEJMra1208943.

Visser, L. C., Ciccozzi, M. M., Sintov, D. J. and Sharpe, A. N. (2019) 'Echocardiographic quantitation of left heart size and function in 122 healthy dogs: A prospective study proposing reference intervals and assessing repeatability', *Journal of Veterinary Internal Medicine*, 33(5), pp. 1909–1920. doi: 10.1111/jvim.15562.

Wernick, M. B., Höpfner, R. M., Francey, T. and Howard, J. (2012) 'Comparison of arterial blood pressure measurements and hypertension scores obtained by use of three indirect measurement devices in hospitalized dogs', *Journal of the American Veterinary Medical Association*, 240(8), pp. 962–968. doi: 10.2460/javma.240.8.962.

Wess, G., Mäurer, J., Simak, J. and Hartmann, K. (2010) 'Use of Simpson's method of disc to detect early echocardiographic changes in Doberman Pinschers with dilated cardiomyopathy', *Journal of Veterinary Internal Medicine*, 24(5), pp. 1069–1076. doi: 10.1111/j.1939-1676.2010.0575.x.

Westphal, G., Garrido, A. D. P. G., De Almeida, D. P., Rocha E Silva, M. and Poli-De-Figueiredo, L. F. (2007) 'Pulse pressure respiratory variation as an early marker of cardiac output fall in experimental hemorrhagic shock', *Artificial Organs*, 31(4), pp. 284–289. doi: 10.1111/j.1525-1594.2007.00377.x.

Wetterslev, M., Møller-Sørensen, H., Johansen, R. R. and Perner, A. (2016) 'Systematic review of cardiac output measurements by echocardiography vs. thermodilution: the techniques are not interchangeable', *Intensive Care Medicine*, 42(8), pp. 1223–1233. doi: 10.1007/s00134-016-4258-y.

Yamashita, K., Ueyama, Y., Miyoshi, K., Igarashi, R., Kushiro, T., Umar, M. A. and Muir, W. W. (2007) 'Minimally Invasive Determination of Cardiac Output by Transthoracic Bioimpedance, Partial Carbon Dioxide Rebreathing, and Transesophageal Doppler Echocardiography in Beagle Dogs', *Journal of Veterinary Medical Science*, 69(1), pp. 43–47. doi: 10.1292/jvms.69.43.

Young, B. C., Prittie, J. E., Fox, P. and Barton, L. J. (2014) 'Decreased central venous oxygen saturation despite normalization of heart rate and blood pressure post shock resuscitation in sick dogs', *Journal of Veterinary Emergency and Critical Care*, 24(2), pp. 154–161. doi: 10.1111/vec.12154.

Zhang, Y., Wang, Y., Shi, J., Hua, Z. and Xu, J. (2019) 'Cardiac output measurements via echocardiography versus thermodilution: A systematic review and meta-analysis', *PLoS ONE*, 14(10). doi: 10.1371/journal.pone.0222105.

Zhang, Z., Xu, X., Ye, S. and Xu, L. (2014) 'Ultrasonographic measurement of the respiratory variation in the inferior vena cava diameter is predictive of fluid responsiveness in critically ill patients: systematic review and meta-analysis.', *Ultrasound in medicine & biology*, 40(5), pp. 845–53. doi: 10.1016/j.ultrasmedbio.2013.12.010.