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DEPARTAMENTO DE MEDICINA VETERINÁRIA

COAGULOPATHY IN SEPSIS AND THE PROGNOSTIC VALUE OF ABNORMAL COAGULATION TIMES

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Orientação | Professora Doutora Sandra Maria da Silva Branco

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To my brightest stars: Schtotsu, Daini, Inu.

To my brightest light, Mew.

To my brightest self; you've made it kiddo!

"It's time to try defying gravity."

– Stephen Schwartz, Defying Gravity

ACKNOWLEDGEMENTS

I would like to show my appreciation and gratitude to those who made this whole crazy journey possible:

To my family for their never-ending support, particularly my brothers Tavinho and Pedro, my Mom, and my Avó. I am also grateful to my godparents, Mika and Orlando, for being my second set of parents and for lending me their laptop when mine decided to take a nap while writing this dissertation.

To my supervisor Prof. Sandra Branco for the ongoing guidance and encouragement, for the prompt availability to help me with my doubts, and for always replying to my emails within minutes.

To my supervisor Dr. Ângela Martins for all the knowledge passed on to me throughout my internship period, for the continuous guidance, for always being a role model of hard work, and for believing in me.

To Prof. Inês Viegas for so eagerly helping me out with the statistical work of this dissertation.

To my internship colleagues and all the staff from Hospital Veterinário da Arrábida that put up with me and my uncontrollable urge to hug the patients; I swear it was all part of the job!

To my hometown friends, who have always been a source of cheerfulness and encouragement. A special thank you goes to my buddies Vasco, Rute, and Raquel, who have been by my side since we were next to toddlers, and to Pat, Inês, and Catarina, who also put up with me just as much.

To my friends who are scattered around the globe for being a source of laughter and enjoyment, even when I am feeling the lowest. A shout out to John for being a sweetheart and reading this dissertation beforehand.

To my friends and colleagues with whom I shared the last 6 years of this veterinary rollercoaster of a course. A special thank you goes to David, Inês, Cláudio, Ana Lurdes, and Flôr for the many moments shared.

To Mafalda and Carolina for keeping my sanity in check throughout one of the toughest times of this journey.

To Diogo for the ongoing support, comfort, and warmth throughout every obstacle.

To my academic family. You lot are amazing individuals, and it is plain silly how much I cherish each and every one of you guys. A special mention goes to my academic godmother Sara and godson Luís. I will make sure to continue to be as clingy as I have always been!

ABSTRACT

Sepsis is a hard to define condition associated with the deleterious systemic inflammatory response syndrome (SIRS) which ultimately leads to the failure of multiple organs. The mediators released throughout this exaggerated inflammatory reaction activate coagulation pathways and generate a dysfunctional response that results in coagulopathy.

The present dissertation includes a literature review regarding the subject of sepsis and associated coagulopathy, along with a study that primarily aims to investigate the use of abnormal coagulation times as biological markers of coagulation dysfunction and as predictors of outcome in veterinary patients who are at risk of developing sepsis.

The results suggest that pairing coagulation time data with an organ failure scoring system may be advantageous in the prediction of outcome. Furthermore, critically ill patients should be given a five-day time frame following admission before euthanasia is considered, as most tend to survive their illnesses once they get past this period.

Keywords: Sepsis; Coagulation; SIRS; Emergency; Critical Care

RESUMO

COAGULOPATIA NA SÉPSIS E O VALOR DE PROGNÓSTICO DE TEMPOS DE COAGULAÇÃO ALTERADOS

A sépsis é uma síndrome de difícil definição e que está associada à síndrome da resposta inflamatória sistémica (SIRS) que leva à falha de múltiplos órgãos. Os mediadores libertados durante esta reação inflamatória exagerada levam à ativação disfuncional da coagulação sanguínea, o que resulta em coagulopatia.

A presente dissertação inclui uma revisão bibliográfica sobre o tema da sépsis e a coagulopatia associada, bem como um estudo cujo objetivo primário é o de investigar a utilização de tempos de coagulação alterados, tanto como marcadores biológicos de disfunção da coagulação sanguínea bem como fatores de prognóstico em pacientes veterinários em risco de sépsis.

Os resultados do estudo realizado mostram vantagem em associar a avaliação dos tempos de coagulação com sistemas de pontuação de falha orgânica para a realização do prognóstico. Estes sugerem também que os pacientes críticos que ultrapassam os primeiros cinco dias após a sua admissão hospitalar tendem a sobreviver.

Palavras-chave: Sépsis; Coagulação; SIRS; Urgências; Cuidados Intensivos

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LIST OF SYMBOLS AND ABBREVIATIONS

ACh – Acetylcholine

AKI – Acute Kidney Injury

ANOVA – Analysis of Variance

ANS – Autonomic Nervous System

AP-1 – Activator Protein 1

aPC – Activated Protein C

aPTT – Activated Partial Thromboplastin Time

ARDS – Acute Respiratory Distress Syndrome

AT – Antithrombin

ATC – Acute Traumatic Coagulopathy

ATP – Adenosine Triphosphate

C1 – Complement Component 1

C3 – Complement Component 3

C5 – Complement Component 5

CARS – Compensatory Anti-inflammatory Response Syndrome

CIRCI – Critical Illness-Related Corticosteroid Insufficiency

CNS – Central Nervous System

d - Days

DAMP – Danger-Associated Molecular Pattern

DIC – Disseminated Intravascular Coagulation

DNA – Deoxyribonucleic Acid

EPCR – Endothelial Protein C Receptor

FV – Factor V or Proaccelerin

FVa – Activated Factor V

FVII – Factor VII or Proconvertin

FVIIa – Activated Factor VII

FVIII – Factor VIII or Antihaemophilic Factor A

FVIIIa – Activated Factor VIII

FIX – Factor IX or Christmas Factor

FIXa – Activated Factor IX

FX – Factor X or Stuart-Prower Factor

FXa – Activated Factor X

FXI – Factor XI or Plasma Thromboplastin Antecedent

FXII – Factor XII or Hageman Factor

FXIII – Factor XIII or Fibrin-Stabilising Factor

FiO_2 – Fraction of Inspired Oxygen

GIT – Gastrointestinal Tract

HPA – Hypothalamic-Pituitary-Adrenal (axis)

HSD – Honest Significant Difference (Tukey's test)

ICU – Intensive Care Unit

IL – Interleukin

IL-1 – Interleukin 1

IL-6 – Interleukin 6

IL-10 – Interleukin 10

LPS – Lipopolysaccharide

MAP – Mean Arterial Pressure

MGCS – Modified Glasgow Coma Scale

MODS – Multiple Organ Dysfunction Syndrome

NF-KB – Nuclear Factor Kappa B

NK – Natural Killer (cell)

NO – Nitric Oxide

PAI – Plasminogen Activator Inhibitor

PAI-1 – Plasminogen Activator Inhibitor Type 1

PAMP – Pathogen-Associated Molecular Pattern

PaO_2 – Partial Pressure of Oxygen

PAR – Protease-Activated Receptor

PIRO – Predisposition, Infection, Response, Organ Dysfunction (sepsis staging system)

PRR – Pattern Recognition Receptor

PT – Prothrombin Time

qSOFA – Quick Sequential Organ Failure Assessment (score)

ROS – Reactive Oxygen Species

Sepsis-1 – 1991 Sepsis Definitions

Sepsis-2 – 2001 Sepsis Definitions

Sepsis-3 – 2016 Sepsis Definitions

SIRS – Systemic Inflammatory Response Syndrome

SOFA – Sequential Organ Failure Assessment (score)

T3 – Triiodothyronine

TAFI – Thrombin-Activatable Fibrinolysis Inhibitor

TF – Tissue Factor

TFPI - Tissue Factor Pathway Inhibitor

Th – T Helper (cell)

Th1 – T Helper 1

Th2 – T Helper 2

Th17 – T Helper 17

TLR – Toll-Like Receptor

TNF – Tumour Necrosis Factor

TNFα - Tumour Necrosis Factor Alpha

TNM – Classification of Malignant Tumours (cancer staging system)

TPA – Tissue Plasminogen Activator

UPA – Urokinase-Type Plasminogen Activator

UPAR – Urokinase-Type Plasminogen Activator Receptor

vWF – von Willebrand Factor

y - Years

PREFACE

The present dissertation was written following a six-month internship, from September 2016 to March 2017, at Hospital Veterinário da Arrábida and Centro de Reabilitação Animal da Arrábida, a small animal hospital connected to a referral rehabilitation centre in the civil parish of Azeitão in Portugal.

Many areas of veterinary medicine were explored throughout this internship, including diagnostics, emergency and critical care medicine, orthopaedic and soft tissue surgery, internal medicine, and small animal rehabilitation. It was during this period that the author determined the topic of his research and began collecting the data that would be subsequently analysed, after developing a particular interest in the subject of sepsis in critically ill patients.

1. LITERATURE REVIEW

1.1. INTRODUCTION TO THE DEFINITION OF SEPSIS

Defining sepsis is not an easy task. The word "sepsis" is as old as ancient Greece when it was originally used to describe decomposition in the presence of bacteria[1], long before anything was known about this serious condition.[2] Prior to 1989, sepsis was merely believed to be associated with bacteraemia.[3]

In 1989, Bone *et al.*[4] defined sepsis syndrome as "the systemic manifestations of presumed sepsis". However, this definition of a systemic response to infection was based on a set of clinical signs which could be found in the absence of infection. This fact led to the creation of the concept of a "Systemic Inflammatory Response Syndrome" (SIRS) in a consensus conference held by the American College of Chest Physicians and the Society of Critical Care Medicine in 1991.[5] SIRS was created to describe the inflammatory response found in sepsis, regardless of its cause. It was established that infection, as well as trauma, pancreatitis, and other non-infectious insults, could trigger this response. It was also suggested that the term sepsis should only be used if SIRS was the result of a confirmed infectious process. SIRS was associated with variables such as altered temperature (hypothermia or hyperthermia), heart rate (bradycardia or tachycardia), respiratory rate (bradypnea or tachypnea), and white blood cell count (leukocytosis or leukopenia), and would be diagnosed if a human patient was positive for at least two of these four criteria.[5] It has later been suggested that dogs should also meet two of these criteria to be diagnosed with SIRS whereas cats would need to fulfil three criteria for the same purpose.[6,7] In addition to the definition of SIRS, the notions of "severe sepsis" and "septic shock" were introduced to describe different stages of sepsis. The concept of a "Multiple Organ Dysfunction Syndrome" (MODS) was also established

to describe the presence of altered organ function in an acutely ill patient such that homeostasis could not be maintained without intervention.[5]

Despite the general acceptance of this new definition of sepsis, many clinicians did not fully agree with it.[8–10] This new approach did not seem to provide a precise definition of sepsis and many considered the SIRS criteria to be too sensitive and nonspecific for its diagnosis since a large number of patients admitted to intensive care units would meet such criteria and would thus be considered septic.[11–16] In 2001, an International Sepsis Definitions Conference was held in an attempt to tackle these issues by revisiting the previous definitions surrounding sepsis.[9] It was recognised that, while still useful, the diagnostic criteria for SIRS were overly sensitive and nonspecific. Thus, a list of additional signs and symptoms of systemic inflammation in response to infection was presented to more accurately reflect the host's clinical response. However, this list was arguably too long to be universally adopted, and the SIRS criteria continued to be used to diagnose sepsis.[2] A conceptual staging system for sepsis called PIRO, inspired by the Classification of Malignant Tumours (TNM) system, was also proposed at this conference as a potential tool for sepsis patient stratification. In the PIRO (an acronym for Predisposition, Infection, Response and Organ dysfunction) model, **P** refers to all predisposing factors which may impact the outcome of sepsis, such as genetic variability, age, the presence of concomitant diseases, and nutritional status. In veterinary patients, racial predisposition would fit into this component. **I** refers to the description of the infection, which includes its etiologic agent, location, and extent. **R** concerns the host's inflammatory response to sepsis. Finally, **O** corresponds to the number of failing organs and the degree of dysfunction. Although promising, PIRO was yet to be fully developed and required further investigation.[9,17–19]

SIRS criteria continued to be criticised for their inadequacy, and the need for a new definition of sepsis remained.[20–23] In 2016, the authors of the Third International Consensus Definitions for Sepsis and Septic Shock released newly updated definitions for sepsis and septic shock. It was suggested that these would be regarded as Sepsis-3, while the 1991 and 2001 versions would be known as Sepsis-1 and Sepsis-2, respectively. Improved understanding of sepsis pathobiology led to its current definition of a "life-threatening organ dysfunction caused by a dysregulated host response to infection".[24]

Furthermore, septic shock was considered a subset of sepsis associated with a higher mortality rate and was characterised by the need for vasopressor therapy to maintain the mean arterial pressure (MAP) of 65 mmHg or more, as well as the presence of serum lactate levels higher than 2 mmol/L despite appropriate fluid resuscitation.[25]

To assess organ dysfunction severity and recognise sepsis in critically ill patients with suspected infection, the authors of Sepsis-3 suggested the use of the Sequential Organ Failure Assessment (SOFA) score. A SOFA score of at least two points is indicative of organ dysfunction and is associated with a higher mortality rate when compared with lower scores. However, SOFA is rather complex and requires laboratory testing, and therefore using it to quickly identify sepsis outside of an intensive care unit (ICU) setting is not realistic. To address this issue, the authors of Sepsis-3 proposed the use of a new straightforward scoring system called "quick SOFA" (qSOFA). This simplified SOFA variant can be used to promptly identify patients with suspected infection who are likely of developing poor outcomes. To determine these patients, qSOFA analyses the existence of altered mentation, hypotension and tachypnea. Each of these clinical signs represents one point, and a score equal to or greater than two points is suggestive of organ dysfunction.[24,26,27]

Controversy has always surrounded the definition of sepsis, and Sepsis-3 is no exception to this as many clinicians are not in full agreement with its foundation.[28–31] Many clinicians believe that the newly recommended criteria for the identification of sepsis require further testing before replacing their antecedents.[32–35] Despite the most recent approach to the definition of sepsis, the SIRS criteria are still considered to be of great utility in the identification of infected patients, as well as any other patients suffering from sterile SIRS.[24,36–39] Sepsis is a very complex condition, and there is yet to exist a widely recognised and gold standard way to identify it.[40]

1.2. THE SYSTEMIC INFLAMMATORY RESPONSE SYNDROME (SIRS)

1.2.1 SIGNS OF SIRS IN VETERINARY PATIENTS

SIRS is a complex and systemic response to an infectious or non-infectious insult that may occur in both human and veterinary patients.[41] The concepts of sepsis and SIRS and all surrounding discussion were initially concerning the human patient. Studies have been conducted in an attempt to adapt the SIRS criteria to veterinary patients and establish limits for each criterion.[6,7] Table 1 shows the suggested criteria, based on such research.

Table 1. Systemic inflammatory response syndrome (SIRS) criteria for dogs and cats (data collected from references 6 and 7).

Clinical Parameters	Dogs (must meet two criteria)	Cats (must meet three criteria)
Heart rate (beats/min)	>120	< 140 or > 225
Respiratory rate (breaths/min)	> 20	> 40
Rectal temperature (C°)	< 38.1 or >39.2	< 37.8 or > 39.7
Leukogram (white blood cells/ µL; % band cells)	<6000 or >16,000; > 3	< 5000 or > 19.500; > 5

The suggested SIRS criteria for cats are slightly different from the ones suggested for dogs. Besides tachycardia, low heart rates are also frequently found amongst critically ill feline patients and should be considered when applying the SIRS criteria. Furthermore, cats must express much higher respiratory rates to be diagnosed with tachypnea, when compared to dogs. Interestingly, it is also suggested that cats must satisfy at least three criteria for the identification of SIRS.[7] Dogs, however, are only required to meet two criteria for SIRS to be identified, much like what happens with human patients.[6] A study

performed by Okano *et al.*[42] suggests that, in canine patients, the prognosis worsens as more SIRS criteria are met. In that same study, the results also indicated that some alterations in the parameters included in the SIRS criteria might be related to worse outcomes when compared to others. Abnormalities in body temperature and white blood cell count seemed to be linked to a poorer prognosis and were considered to be the most reliable of the four parameters to be evaluated since external stimuli can easily influence both respiratory and heart rates. Contrary to these findings, a study by Declue *et al.*[43], performed with cats, revealed that the number of satisfied SIRS criteria was not correlated with prognosis. The dissonance in the results of reports such as these shows how dogs and cats can respond differently to inflammation and sepsis and should not be evaluated as being part of the same species.

Infection, heat stroke, pancreatitis, immune-mediated disease, neoplasia, trauma, and burns are the most common causes of SIRS in veterinary patients. Clinical signs of SIRS are usually nonspecific and can change depending on the underlying disease process. They tend to mimic the manifestations of sepsis and are generally treated similarly.[44] It is important to mention that dogs and humans tend to display clinical signs of an initial hyperdynamic phase of sepsis such as loss of appetite, depression, hyperemic mucous membranes, bounding peripheral pulses, tachycardia, tachypnea, and fever. Cats, however, rarely manifest this hyperdynamic state. Thus, the clinical signs of sepsis found in cats tend to be related to a secondary hypodynamic phase and may include lethargy, diffuse abdominal pain, pale mucous membranes, tachypnea, bradycardia, hypotension and hypothermia. Cats are also more likely to experience hypotension, hypoglycaemia and hyperbilirubinemia than dogs.[7,44,45]

Blood cell count alterations, such as neutrophilic leukocytosis, and toxic cytologic changes of the neutrophils are common in patients with SIRS, as well as a variety of other changes on a biochemical level. Blood glucose levels tend to fluctuate between hyperglycaemia in the early phase of inflammation when gluconeogenesis is increased, and subsequent hypoglycaemia once glucose levels drop as a result of excessive use. Albumin concentration levels are likely to drop secondarily to reduced albumin production by the liver, in favour of acute phase proteins. Changes in endothelial permeability found in SIRS also lead to plasma protein leakage and consequently loss of

albumin.[44] The resulting hypoalbuminemia may cause the development of pulmonary and peripheral oedema, which was evident in a study performed with cats suffering from sepsis, by Brady *et al.*[7] Liver enzymes, such as alanine aminotransferase and aspartate aminotransferase, are inclined to increase in concentration due to changes in perfusion and decreased tissue oxygenation. Serum bilirubin may also suffer alterations, usually as a result of cholestasis.[44] Haemolysis may also be responsible for icterus in cats with sepsis considering how common anaemia seems to be present in these patients.[7] A study by Schaefer *et al.*[46] showed that proteinuria is also present in dogs with SIRS, as a result of altered urinary protein excretion due to glomerular and tubular malfunction.

1.2.2. PATHOPHYSIOLOGY OF SIRS

In ancient Rome, Celsus was the first to introduce the four signs widely used to describe an inflammatory response: redness (*rubor*), swelling (*tumor*), heat (*calor*), and pain (*dolor*). Many centuries later, a fifth sign, loss of function (*function laesa*), was added to this list.[47,48] These terms characterise the visual changes that occur in a localised inflammatory response to tissue damage or infection.[49] Local blood vessel dilation and increased permeability result in the passage of an additional number of erythrocytes and fluids into the damaged area resulting in redness, heat, and swelling. Cells also infiltrate into the affected area, and prolonged inflammatory responses may generate deposits of connective tissue, further increasing the swelling. Resulting oedema leads to the stretching of sensory nerves, which results in pain. Pain is also a consequence of the initial tissue damage as well as the resulting inflammatory response itself and the effects of its mediators. Loss of mobility in structures such as the joints, due to pain and oedema, and replacement of once functional cells with scar tissue are examples of circumstances that lead to loss of function.[48]

The local hemodynamic changes in the inflammatory response are aimed at defending the host and eliminating harmful agents and damaged cells.[49] Thus, localised inflammation is a physiological protective response, controlled by inflammatory mediators. However, overactivation of this inflammatory reaction or loss of its local control may result in the exaggerated systemic response we know as SIRS.[50]

SIRS is a dysregulated inflammatory response to injury or microbial invasion. Even though this syndrome is an essential part of sepsis when triggered by infectious agents, it can also occur in the absence of infection. Regardless of the initial insult, the resulting inflammatory response is considered to be fairly similar.[51] When infection is the cause of SIRS, both gram-negative and gram-positive bacteria, as well as parasitic, fungal, protozoan and viral microorganisms, can be responsible for inciting the systemic response (Figure 1).[52] However, infections caused by gram-negative bacteria seem to be both the most prevalent and dangerous, in cases of sepsis.[53,54] *Escherichia coli* is the most commonly isolated microorganism in dogs and cats with sepsis.[7,55–60] Interestingly, in the particular case of sepsis associated with pyothorax, members of the genus *Pasteurella* appear to be more commonly isolated in cats amongst facultative bacteria, whereas *Escherichia coli* continues to be more prevalent in dogs.[61,62]

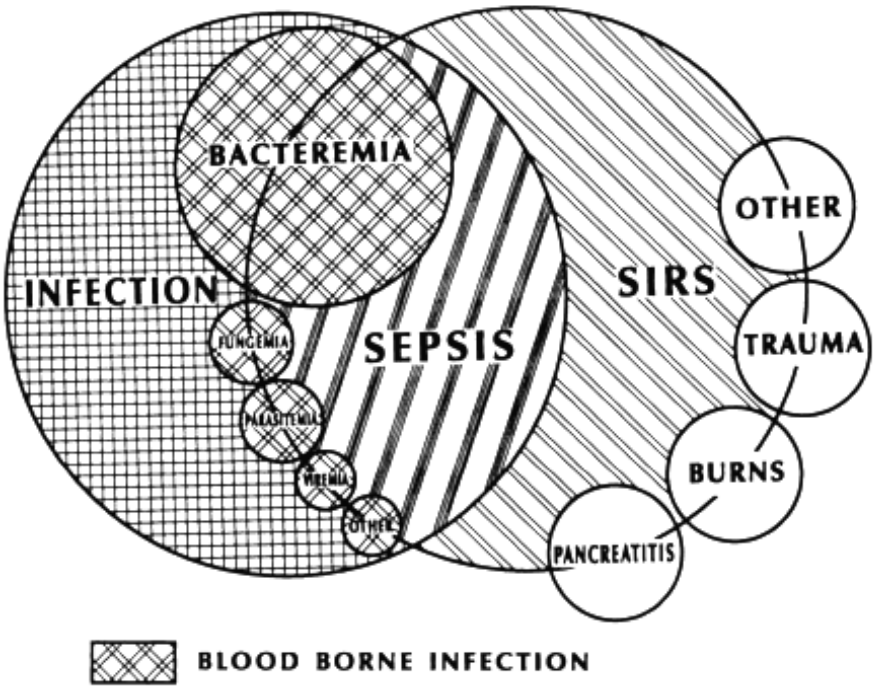

Figure 1. The interrelationship between the systemic inflammatory response syndrome (SIRS), infection, and sepsis (reprinted from reference 5 with permission from Elsevier).

In human patients, the infectious processes that represent the most common causes of sepsis are pneumonia, urinary tract infections, intra-abdominal infections, and bacteraemia.[63] In dogs, sepsis has been linked with conditions such as septic peritonitis, pancreatitis, pneumonia, pyometra, prostatitis, and wound infections.[6,57] In our domestic felines, sepsis has been associated with conditions including septic peritonitis, pneumonia, bacteraemia, endocarditis, pyelonephritis, hepatic abscessation, and pyothorax.[7,58,59,62]

1.2.2.1. PATHOGEN AND TISSUE DAMAGE RECOGNITION

Mammals, such as humans and their small animal companions, possess an immune system with the task of protecting them against the invasion of harmful microorganisms. This immune system includes both innate and acquired immunity. While the innate immune system represents the first line of host defence against infection, the acquired immune system is associated with later phases of pathogen elimination and with the development of immunological memory.[52] The innate immune system is responsible for containing the infection and delivering antigens to local lymph nodes, which results in the activation of the acquired immune system and consequent eradication of infection.[64] For an invading microorganism to be able to successfully disseminate and cause sepsis and septic shock, both innate and acquired immune defences must be breached.[65,66]

The innate immune system includes the activity of many different cells such as macrophages, neutrophils, natural killer cells (NK), endothelial and epithelial cells, and dendritic cells.[52,67,68] These cells can detect the presence of molecular structures associated with microbial pathogens and tissue damage, as well as endogenous molecules released during cellular injury, through a group of surface proteins named pattern recognition receptors (PRRs).[67–69] Many of these PRRs have been identified and extensively studied, and one of the best-understood families of PRRs is the Toll-like receptors (TLRs) family.[70,71]

PRRs, such as TLRs, can recognise particular components expressed by microorganisms known as pathogen-associated molecular patterns (PAMPs), as well as endogenous mediators released during tissue injury and cell death known as “alarmins” or danger-

associated molecular patterns (DAMPs).[66,67,71] Some authors seem to consider that the term DAMPs includes both PAMPs and alarmins [69,72], but the previous distinction will be the one used in the present dissertation.

Cell wall components, such as lipopolysaccharide (LPS) expressed by gram-negative bacteria (one of the most potent PAMPs), flagellin, and bacterial deoxyribonucleic acid (DNA) are some examples of PAMPs, which tend to be closely related to the survival or pathogenicity of the invading microorganism.[68] Examples of DAMPs include heat shock proteins, fibrinogen, hyaluronic acid, and components of the endothelial glycocalyx.[52,73,74]

The recognition of PAMPs and DAMPs by PRRs results in the activation of the cell through a downstream of signalling cascades that culminate in a transcriptional response, via the mobilisation of transcription factors such as nuclear factor-kappa B (NF-kB) and activator protein 1 (AP-1). This cell activation results in the production and secretion of inflammatory mediators like cytokines, chemokines and complement-activating products.[68,71,75–80] Figure 2 exemplifies this response.

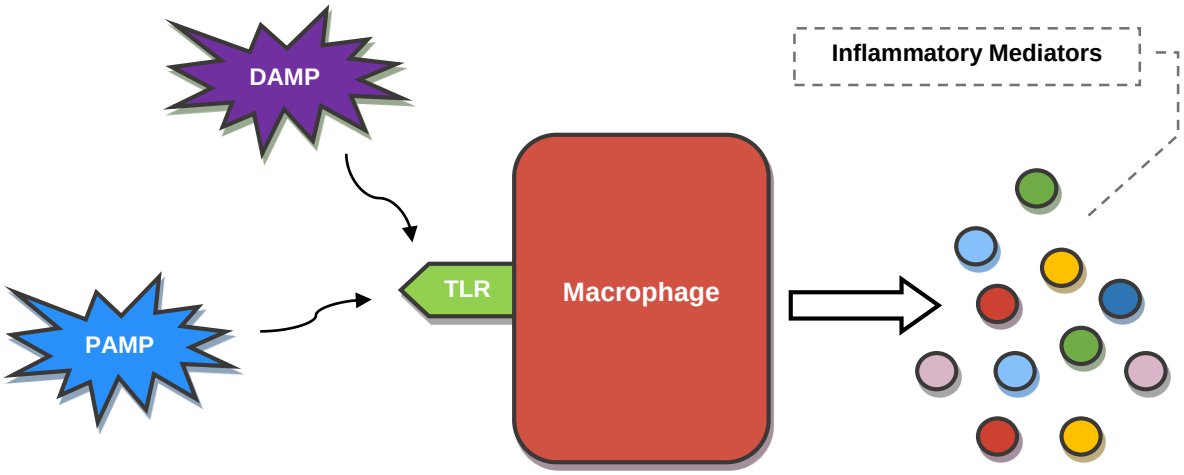

Figure 2. Recognition of infection or tissue injury by a macrophage (original figure). DAMP, Danger-associated molecular pattern; PAMP, pathogen-associated molecular pattern; TLR, Toll-like receptor.

1.2.2.2. THE HYPERINFLAMMATORY RESPONSE IN SIRS

Cytokines are small protein mediators of low molecular weight (usually less than 40 kDa) that initiate, modulate, and sustain inflammatory interactions.[76,78] The main proinflammatory cytokines responsible for inducing a systemic inflammatory response are those of the tumour necrosis factor (TNF) family and some interleukins (ILs), namely tumour necrosis factor alpha (TNFα), interleukin 1 (IL-1), and interleukin 6 (IL-6).[68,71,79]

Once released into circulation, these cytokines will signal endothelial cells to upregulate adhesion molecules that promote the migration of leukocytes from the microcirculation into sites of tissue injury or infection, recruiting them to perform the phagocytosis of pathogens and removal of damaged and dead host cells. [81–83] This proinflammatory environment leads to the secretion of additional cytokines as well as secondary mediators such as nitric oxide (NO), reactive oxygen species (ROS), and lipid factors.[64,77,84] Under controlled inflammatory responses, this process would ultimately result in the clearance of infection and tissue healing.[67,80]

During SIRS, there is an overstimulation of immune cells as a response to extremely high levels of DAMPs from injured host tissue or PAMPs from invading microorganisms.[77] This leads to an uncontrolled production and secretion of proinflammatory mediators, also known as “cytokine storm”, that enter the systemic circulation and travel to organs distant to the initial site of tissue damage or infection, resulting in the global activation of the inflammatory system.[80,85]

The acquired immune system is also involved in the production of cytokines and development of SIRS and sepsis.[85,86] Antigen-presenting cells, such as monocytes and dendritic cells, activate the acquired immune response by interacting with naïve T cells and driving them to proliferate and differentiate into T helper (Th) cells. T helper 1 (Th1) and T helper 17 (Th17) cells are responsible for producing additional proinflammatory cytokines whereas T helper 2 (Th2) cells produce anti-inflammatory cytokines. Shifts in the balance between Th1/Th17 and Th2 cells dictate the nature of the immune response.[85–87] Early stages of SIRS have been associated with increased proinflammatory cytokine production while anti-inflammatory activity and immune suppression are more characteristic of later phases of the syndrome.[86]

The autonomic nervous system (ANS) takes part in the inflammatory response as well. Immune cells are capable of producing and secreting neurotransmitters, as well as expressing receptors for such mediators, allowing the nervous and immune systems to communicate during inflammation.[77] Released cytokines also provide the central nervous system (CNS) with updated information regarding the ongoing inflammatory response.[88] Vagus nerve stimulation triggered by inflammatory stimuli has been shown to suppress inflammation.[89,90] Efferent activity in the vagus nerve results in acetylcholine (ACh) secretion in organs of the reticuloendothelial system such as the liver, heart, spleen, and gastrointestinal tract. Exposure of tissue macrophages to ACh inhibits the release of proinflammatory cytokines. This anti-inflammatory mechanism is called the "cholinergic anti-inflammatory pathway" and is an important part of the "inflammatory reflex" carried out by the nervous system to control acute inflammation.[91,92] Failure of mechanisms such as these due to CNS dysfunction in SIRS may contribute to the exacerbation of the inflammatory response.[88] Furthermore, some authors have suggested that the release of catecholamines by phagocytes and cells of the sympathetic branch of the ANS, in early phases of the syndrome, may amplify the proinflammatory responses of macrophages, neutrophils and dendritic cells. However, this subject appears to be controversial and not yet fully understood.[77,93–95]

The hyperinflammatory response developed in SIRS is further aggravated by the systemic activation of the complement system, which results in the generation of large amounts of proinflammatory peptides that act as leukocyte chemoattractants, enhance adhesion molecule expression, increase vascular permeability, and stimulate cytokine production.[77,84,96,97] Excessive complement activation has also been previously linked to neutrophil dysfunction and increased mortality in cases of severe trauma.[98,99]

1.2.2.3. THE COMPENSATORY ANTI-INFLAMMATORY RESPONSE SYNDROME (CARS)

Following the recognition of PAMPs and DAMPS, proinflammatory cytokines are not the only ones to be released. In fact, anti-inflammatory cytokines, such as interleukin 10

(IL-10), and proinflammatory cytokine receptor antagonists are also secreted by immune cells in an attempt to control the resulting inflammatory response and prevent it from becoming excessive and causing damage.[100,101] In SIRS, however, this regulatory mechanism is overwhelmed, and the development of the exaggerated proinflammatory response takes place.[49,102] Following the systemic inflammation generated in SIRS, an opposing exaggerated anti-inflammatory response may also develop, leading the organism to a state of "immune paralysis" and to what is known as the compensatory anti-inflammatory response syndrome (CARS).[102–106] Many patients that survive the initial hyperinflammatory phase of SIRS may later succumb to the effects of this status of immunological depression.[107,108]

There is a large number of phenomena that contribute to the development of CARS, but like many other topics surrounding SIRS and sepsis, a great deal of them are still under research.[104–106,109] One of the hallmarks of CARS is the depletion of many types of immune cells via dysregulated apoptosis induced by mediators such as TNFα, IL-1, IL-6, NO and ROS.[66,110–114] An adjusted version of this interaction would represent a regulatory mechanism to mediate inflammatory responses. Following SIRS, however, it ends up resulting in the death of a lot of immune cells, rendering the organism unprotected against secondary infections.[64,111,115] Many other types of cells such as neurons, epithelial and endothelial cells, thymocytes, and cardiac myocytes also display accelerated apoptosis during systemic inflammation.[111,116] Additionally, this increased level of apoptosis stimulates some of the remaining immune cells to secrete anti-inflammatory cytokines such as IL-10.[113]

An overall increased production of IL-10 is characteristic of CARS.[100,105,106] High levels of this cytokine are responsible for decreasing proinflammatory cytokine synthesis by Th1 cells, monocytes, neutrophils, and dendritic cells, as well as inhibiting monocytes of their ability to present antigens and activate cells of the acquired immune system.[113,117–122] Following systemic inflammation, there is also an increase in the number and suppression ability of regulatory T cells. These cells are a subpopulation of T cells that contribute to the development of CARS by reducing Th1 proliferation and inducing further apoptosis of monocytes and neutrophils.[68,101,113,123] Interactions such as these encourage the shift towards a Th2 predominant response which results in the release of

additional IL-10 and other anti-inflammatory cytokines, further boosting immunosuppression.[101,113] The CNS may also contribute to the development of CARS by inhibiting the release of proinflammatory cytokines by macrophages through the previously mentioned cholinergic anti-inflammatory pathway.[91,92,101,109] Catecholamines and cortisol released as a result of the activation of the hypothalamic-pituitary-adrenal (HPA) axis, triggered by SIRS, also contribute to the shift towards Th2 predominance by inhibiting Th1 cytokine synthesis and upregulating Th2 cytokine production.[88,106,113,124–126]

Throughout the years, many theories have been made regarding the interactions between the hyperinflammatory and hypoinflammatory states observed in SIRS.[102,103,127] Current models of SIRS suggest the occurrence of a cycle between each state with both contributing to patient morbidity and mortality.[107,108,128] The development of secondary infections may be responsible for the generation of new proinflammatory responses and thus, the longer SIRS goes on, the more likely a patient is to experience profound immunosuppression.[107] Regardless of which state is predominant, it appears that both proinflammatory and anti-inflammatory responses are concurrently active during the syndrome.[107,108,127,128]

1.2.2.4. SEPTIC SHOCK

Septic shock is the most severe form of sepsis.[129,130] According to its most recent definition, septic shock is considered "a subset of sepsis in which underlying circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than sepsis alone".[25] As mentioned earlier, septic shock involves persistent hypotension and is characterised by the need for vasopressor therapy to maintain the minimum MAP levels of 65 mmHg, as well as the presence of a serum lactate level greater than 2 mmol/L, despite adequate fluid resuscitation.[25]

The excessive release of cytokines during early stages of sepsis leads to vascular changes, such as peripheral vasodilation and increased permeability of capillaries, that promote loss of intravascular fluid, reduced systemic vascular resistance, and decreased venous return and preload. To maintain perfusion as a response to these hemodynamic changes,

heart rate and stroke volume increase. This hemodynamic instability is what characterises the initial hyperdynamic phase of SIRS.[51,81]

As the syndrome progresses, widespread microvascular thrombosis develops, further hindering blood flow and tissue oxygenation.[81,131,132] Ultimately, this hemodynamic instability evolves into myocardial depression, followed by cardiovascular collapse, the establishment of the hypodynamic phase of SIRS, and the development of septic shock (Figure 3).[51,133] Systemic oxygen delivery becomes insufficient to meet the demands of the tissues and generalised tissue hypoxia occurs, leading to the increased production of lactate due to anaerobic cellular respiration.[134,135] The resulting tissue hypoxia is a consequence of generalised inflammation, and it may also further amplify the inflammatory response by inducing the production of additional proinflammatory cytokines.[136]

Thus, septic shock is a complex type of shock that not only includes elements of distributive shock due to increased vascular permeability but also of hypovolemic and cardiogenic shock as a result of peripheral vasodilation and reduced cardiac output. [81]

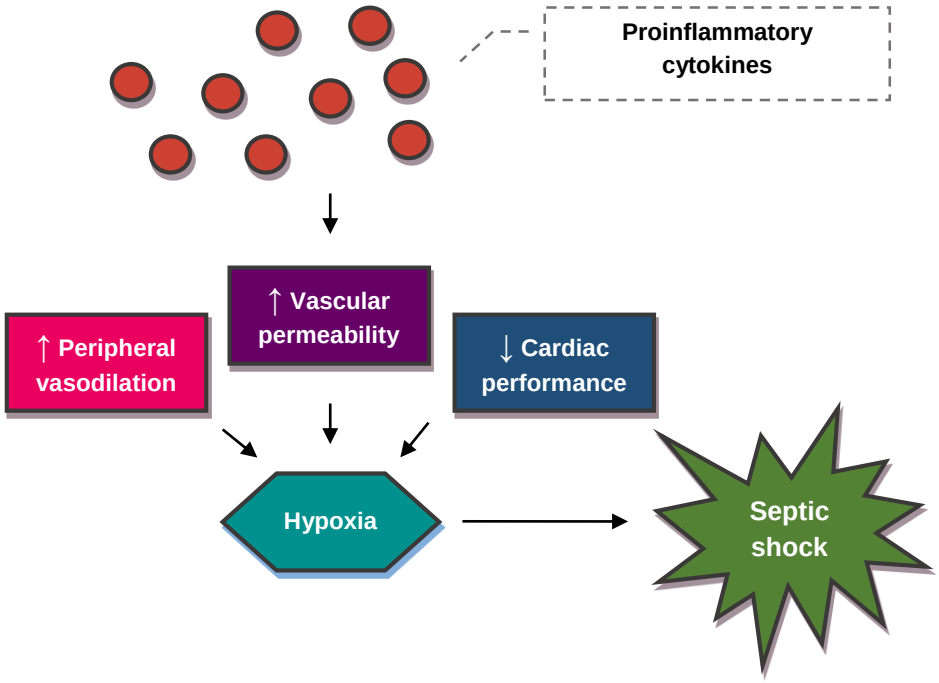

Figure 3. The pathophysiology of septic shock (original figure).

1.2.2.5. THE MULTIPLE ORGAN DYSFUNCTION SYNDROME (MODS)

The multiple organ dysfunction syndrome (MODS) is the ultimate sequela of SIRS and represents an increased risk of death.[49,132,137] In fact, Kenney *et al.*[138] have shown that the mortality rate of canine patients with sepsis suffers an increase for each additional dysfunctional organ system. MODS is characterised by the need of intervention to maintain homeostasis, which would be otherwise accomplished by adequate organ function.[5]

When organ dysfunction is the outcome of a systemic inflammatory reaction, the resulting phenomenon is classified as secondary MODS for the reason that its development is a consequence of the host's response to an insult. However, MODS can also be the direct result of the damage caused by the insult itself. In this case, the syndrome is identified as primary MODS, and it tends to unfold rather quickly.[5] For example, a patient that has been hit by a moving vehicle may quickly develop acute lung injury as a result of traumatic pulmonary contusion. If this is not the case, the inflammatory reaction caused by the incident itself may become excessive and cause damage to the lungs, as well as to other organs.[137]

The pathogenesis of secondary MODS is not entirely understood, but there appear to be many contributing factors to the development of organ failure.[139,140] The hemodynamic changes resulting from the dysregulated inflammatory response in SIRS play a major role in the promotion of organ damage.[82,137,140] Diminished tissue perfusion as a result of microvascular dysfunction and thrombosis leads to tissue hypoxia and cell death, which added to the increased apoptosis observed in SIRS, results in both organ damage and the release of additional DAMPs that perpetuate the inflammatory process.[116,132,139,141,142] Neutrophils that are recruited and activated during SIRS also contribute to the development of organ damage, not only by secreting additional inflammatory mediators that potentiate the inflammatory response but also by causing local tissue damage through the release of ROS and proteolytic enzymes.[82,140,142]

Mitochondrial dysfunction is considered to be highly involved in the pathogenesis of MODS.[143,144] Generalised tissue hypoxia resulting from an exaggerated inflammatory response may compromise the mitochondrial function of generating adenosine triphosphate (ATP). Furthermore, the excessive amount of NO and ROS in circulation can cause direct damage to mitochondrial structures, such as the lipid membrane, and suppress mitochondrial respiration and ATP synthesis.[145] Low levels of triiodothyronine (T3) resulting from thyroid dysfunction in critical illness are also believed to have an adverse impact on mitochondrial activity.[145,146] Cell death occurs in the absence of ATP and with it the eventual alteration of organ function.[144,145] Interestingly, the mitochondrial dysfunction caused by ROS seems to trigger the production of additional ROS by the mitochondria themselves, further amplifying the oxidative damage caused.[147] Additionally, damaging the mitochondria perpetuates the inflammatory response due to mitochondrial DNA being released and acting as a DAMP.[84,148]

Clinical signs of dysfunction of the gastrointestinal tract (GIT) include changes in appetite such as hyporexia and anorexia, as well as vomiting, gastric ulceration, and diarrhoea.[137,149] However, a dysfunctional GIT may also contribute to MODS through the phenomenon of bacterial translocation.[150–152] Bacterial translocation is the passage of bacteria or antigenic macromolecules from the GIT to normally sterile tissues and organs, through the intestinal mucosal barrier.[153,154] Reduced oxygen delivery to the GIT, once again as a result of the hemodynamic changes induced by SIRS, culminates in intestinal ischemia, epithelial cell injury and apoptosis, and increased intestinal permeability.[155–157] Additionally, hypoperfusion of the GIT results in reduced intestinal motility that promotes bacterial overgrowth.[158,159] Furthermore, the absence of luminal nutrients due to undernutrition in critically ill patients further compromises the functional and structural integrity of the intestinal epithelium.[156,160] Both the dysfunction of the intestinal barrier and bacterial overgrowth, as well as the presence of a dysfunctional immune system, favour bacterial translocation.[152–155,161]

Early theories regarding bacterial translocation suggested that bacteria would reach the systemic circulation solely via the portal vein. However, this hypothesis was eventually rejected as new conflicting data emerged.[162–164] It is currently believed that according to the “gut-lymph hypothesis”, the translocating bacteria and bacterial products are exposed

to intestinal immune cells and stimulate the release of inflammatory mediators.[151,157,164] Although the majority of bacteria suffer phagocytosis and contribute to this local inflammatory response, a small number of translocated bacteria survive and become trapped in the intestinal lymph nodes, where additional inflammatory reactions are induced.[161,165] Surviving bacteria, cell wall fragments and protein components of the dead bacteria, and cytokines and chemokines generated in the GIT then travel through the mesenteric lymphatics to the cisterna chyli and are released into systemic circulation via the thoracic duct. These products initially reach the pulmonary circulation and activate the alveolar macrophages. The end result of this process is the development of acute lung injury, along with the intensification of systemic proinflammatory activity and MODS.[151,153]

Interestingly, the lungs are common targets of organ damage in patients with MODS.[137] Pulmonary damage as a result of a deleterious inflammatory response often leads to the development of acute lung injury, followed by its most severe presentation, the acute respiratory distress syndrome (ARDS).[166] The process through which systemic inflammation promotes ARDS involves the infiltration of activated neutrophils into the pulmonary interstitium and alveolus, epithelial and endothelial cell damage and apoptosis, and increased microvascular permeability, followed by pulmonary oedema, atelectasis and interstitial fibrosis.[111,139,140,167] Clinical signs of this pulmonary dysfunction may include respiratory distress, tachypnea, progressive hypoxemia, and cyanosis.[166]

Dysfunction of the liver may also be observed in patients with MODS. Hepatic injury contributes to the establishment of hypoglycaemia as a result of reduced gluconeogenesis and glycogenolysis. Protein synthesis, along with lactate and amino acid clearance, also become decreased following hepatic dysfunction.[168] Furthermore, activated Kupffer cells are responsible for producing a variety of inflammatory mediators that end up contributing to the local and systemic inflammatory responses.[140,168] The main manifestations of hepatic dysfunction tend to be hyperbilirubinemia, as a result of intrahepatic cholestasis, and elevated levels of serum aminotransferases.[137,168]

Cardiac dysfunction in MODS is often present in the form of myocardial depression.[139] The mechanism that leads to the development of myocardial depression is incompletely

understood and appears to be multifactorial.[140,169] In addition to the previously mentioned harmful effects of systemic inflammation, cardiac dysfunction might be associated with alterations in calcium physiology, sympathetic overstimulation, and the presence of circulating myocardial depressant substances that are yet to be fully identified.[137,169–171] Manifestations of cardiac dysfunction may include hypotension despite fluid resuscitation, presence of arrhythmias, and tachycardia.[137,140,169] Cats may also uniquely display bradycardia, which is thought to be the consequence of increased vagal tone or cytokine-associated myocardial depression.[58]

The main phenomenon contributing to the development of acute kidney injury (AKI) and subsequent kidney dysfunction in MODS seems to be the increased epithelial cell apoptosis induced by inflammatory cytokines, whereas renal epithelium necrosis as a result of renal hypoperfusion appears to be less common.[84,116,140] Not only does renal dysfunction promote an increase in serum creatinine concentration values but it may also contribute to the development of neurologic dysfunction.[172] The process behind the dysfunction of the CNS is rather complex and involves the activation of cerebral endothelial cells and consequent alteration of the blood-brain barrier. The disruption of the blood-brain barrier causes the release of a variety of mediators into the brain that contributes to the activation of microglial cells, which are the local immune cells. These are then responsible for releasing proinflammatory mediators such as cytokines, NO, and ROS which cause local injury and perpetuate the dysfunction of the blood-brain barrier.[173] Encephalopathy and peripheral neuropathy are the repercussions of CNS damage in MODS, as well as the deterioration of the mental statuses of the affected patients.[137,167]

Another sequela of SIRS is the occurrence of critical illness-related corticosteroid insufficiency (CIRCI) due to the dysfunction of the HPA axis and subsequent adrenal insufficiency.[126,174–176] The HPA axis is activated in response to the stress caused by the systemic inflammatory insult.[177] Activation of the HPA axis ultimately leads to increased cortisol release from the adrenal cortex.[176] This increase in cortisol production is important in the organism's adaptation to illness and the magnitude of its release tends to be proportional to the severity of stress.[146,176,178] Cortisol contributes to the maintenance of adequate perfusion to the vital organs by aiding in the modulation of the immune response and in the preservation of vascular reactivity to circulating

catecholamines.[135,146,176] However, this response weakens as SIRS progresses, resulting in reduced adrenal function and the establishment of CIRCI.[126,179] CIRCI represents the inadequacy in corticosteroid activity for the severity of a patient's illness, and it can be the result of adrenal failure or tissue resistance to corticosteroids.[176,180,181] Even though CIRCI tends to disappear with the resolution of SIRS, it is possible that some patients develop long-term adrenal insufficiency due to structural damage to the adrenal glands as a result of haemorrhage and ischemia.[146,180] CIRCI can lead to further hemodynamic instability along with persistent hypotension.[41,146]

1.3. COAGULOPATHY IN SEPSIS

Sepsis is associated with haemostatic abnormalities resulting from the dysfunctional activation of blood coagulation throughout the process of systemic inflammation.[83,140,182] The promotion of clotting observed in SIRS results in coagulation abnormalities that range from subclinical clot formation to widespread microvascular thrombosis and haemorrhage which are typical of disseminated intravascular coagulation (DIC).[183,184] The coagulation disorders that accompany sepsis are major contributors to the development of MODS and are thus associated with increased mortality.[83,132,137,140,185]

The systemic inflammatory response present in SIRS is responsible for inducing dysfunctional coagulation through three primary mechanisms: increased activation of blood coagulation, impairment of anticoagulant mechanisms and suppression of fibrinolysis.[128,182,185,186]

1.3.1. FROM SYSTEMIC INFLAMMATION TO THE ACTIVATION OF BLOOD COAGULATION

Coagulation used to be traditionally described through a cascade model involving independent intrinsic and extrinsic pathways. At the present time, however, this classification is deemed outdated, and a newer cell-based model is considered to offer a

better description of the coagulation process. This contemporary model describes coagulation through three different phases: initiation, amplification, and propagation.[187–191]

Tissue factor (TF) is a 47 kDa transmembrane glycoprotein whose expression plays a central role in the activation of blood coagulation in sepsis.[132,192] The disruption of vascular integrity caused by inflammation leads to the exposure of TF in cells which are not in circulation or direct contact with blood.[186,193] Furthermore, cytokines released throughout the systemic inflammatory process, such as TNFα, IL-1, and IL-6, are responsible for inducing endothelial, immune, and various other cell types to express TF.[132,186,194–196]

Once exposed to the bloodstream, TF binds to circulating coagulation factor VII (FVII), also known as proconvertin, converting it to its active form (FVIIa) and generating an active TF-FVIIa complex.[191,193,197] This complex is then responsible for activating factor IX (FIX), also called Christmas factor, to FIXa, and factor X (FX), also known as Stuart-Prower factor, to FXa.[190,191,198] FIXa also further activates FX by interacting with factor VIII (FVIII), also named antihaemophilic factor A, in its active form (FVIIIa).[191,197] In turn, FXa forms a complex with factor V (FV), or proaccelerin, in its active form (FVa). The formed complex is then responsible for inducing the cleaving of prothrombin to thrombin.[189,191,193,197] The aforementioned process represents the initiation phase of coagulation.[187,189–191]

FXa is capable of generating a small amount of thrombin by itself, which in turn is responsible for activating FV and FVIII and subsequently bolstering further thrombin production.[190,191,199] Initially generated thrombin activates nearby platelets, which are essential in the amplification of the coagulation process.[190,193,199,200] During the inflammatory response, exposed collagen as well as circulating endotoxin and proinflammatory mediators, such as platelet-activating factor, may also activate platelets.[186,187,190,193,200,201] The activation of a platelet leads to the expression of P-selectin on its membrane. Similarly, activated endothelial cells also express P-selectin. P-selectin is a glycoprotein that mediates the adherence of platelets to endothelial cells and leukocytes, which helps to localise thrombus formation. Additionally, these interactions lead to further NF-kB activation and monocyte TF expression.[186,189,193,201] Activated

platelets and endothelial cells also release a glycoprotein called von Willebrand factor (vWF) which enhances both platelet aggregation and adherence to the site of injury.[77,189] As the platelet aggregate grows, a temporary platelet plug is formed.[191,200] Once this localised plug is established, the activated platelets augment thrombin generation by providing a procoagulant phospholipid surface on which thrombin can convert FV to FVa and FVIII, which is initially bound to vWF, to FVIIIa.[190,191,193,201] Calcium acts as a cofactor in many interactions throughout the coagulation process by facilitating coagulation factor assembly on phospholipid membranes, such as those of activated platelets.[189,191,197,199,202,203]

The activation of platelets and generation of FVa and FVIIIa represent the amplification phase of the coagulation process, whereas the resulting increased thrombin generation represents the propagation phase (Figure 4).[187,189–191]

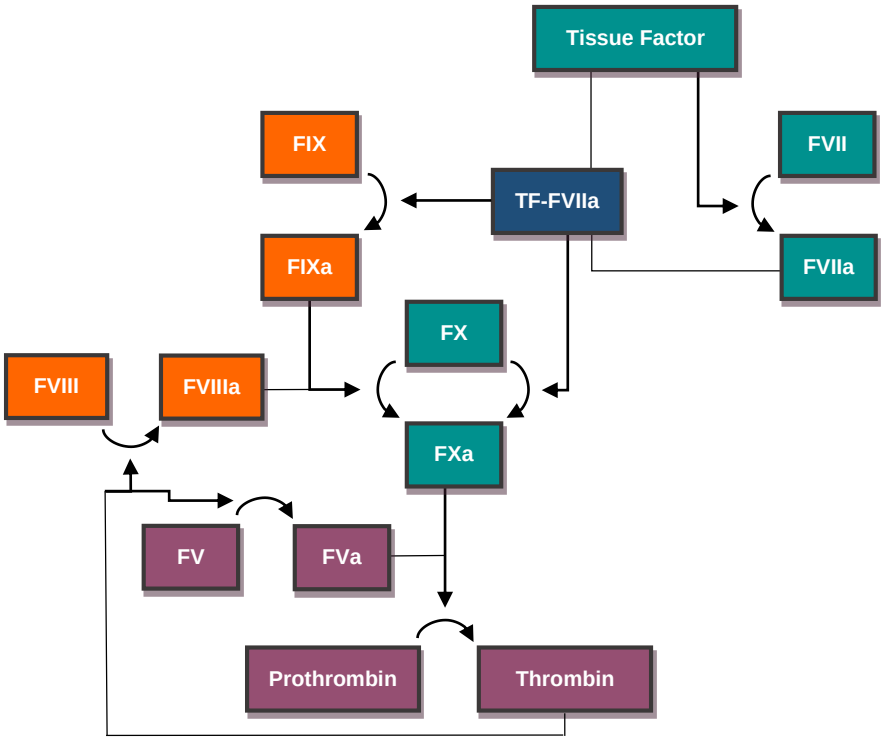

Figure 4. The current concept of coagulation in sepsis (original figure). Tissue factor forms a complex with factor VIIa (FVIIa) that ultimately leads to the generation of trace amounts of thrombin. The generated thrombin then activates factor V (FV) and factor VIII (FVIII) on the membrane of activated platelets, which results in a substantial increase in thrombin production.[190,191,197]

The propagation phase results in the generation of a burst of thrombin that causes the conversion of fibrinogen to fibrin.[187,189] Thrombin additionally activates factor XIII (FXIII), also known as fibrin-stabilising factor, whose function is to cross-link the fibrin now incorporated in the platelet plug, granting it enhanced strength and stability. Furthermore, thrombin activates the thrombin-activatable fibrinolysis inhibitor (TAFI), an enzyme that helps prevent the fibrinolysis of the newly formed thrombus.[187,191]

1.3.2. IMPAIRMENT OF ANTICOAGULANT MECHANISMS

Physiological anticoagulant pathways exist to prevent blood coagulation from becoming excessively activated. During systemic inflammation, however, these mechanisms may become suppressed. There are three main antithrombotic mechanisms through which procoagulant activity is regulated. These include the anticoagulant activity of the tissue factor pathway inhibitor (TFPI), protein C, and antithrombin (AT).[73,188,193,204]

The majority of TFPI is bound to the microvascular endothelium. Smaller amounts of this glycoprotein can also be found in circulation, either bound to plasma lipoproteins or in free form, and within the cytoplasm of platelets.[203,205–207] TFPI is released in response to thrombin and other stimulants. Interestingly, heparin is a potent inducer of TFPI release.[205,207,208] TFPI inhibits the production of thrombin by binding to and inactivating FXa and the TF-FVIIa complex.[83,207,209,210] In sepsis, the production of TF that accompanies the systemic inflammatory response appears to overwhelm the generation of TFPI, thus promoting a procoagulant state.[132,206,211–213] Furthermore, an enzyme called neutrophil elastase, which is released by activated neutrophils during inflammation, is responsible for causing the proteolysis of TFPI, preventing it from inactivating FXa and the TF-FVIIa complex.[212,214,215] Studies with animal models have shown that both the administration of TFPI and the inhibition of neutrophil elastase, in sepsis, were associated with improved survival.[216,217]

Protein C is a circulating glycoprotein which is activated by thrombin. Once activated, protein C degrades FVa and FVIIIa, limiting further thrombin generation.[189,191,193,203,218] Additionally, thrombin complexes with a transmembrane receptor present on endothelial

cells named thrombomodulin. The creation of this complex enhances protein C activation which leads to a substantial increase in the generation of activated protein C (aPC).[189,191,203,219] Protein C activation is further amplified by the presence of another receptor found on the membrane of endothelial cells, the endothelial protein C receptor (EPCR), that binds to it and optimally presents it to the complex formed between thrombin and thrombomodulin.[193,203] The combination of protein C consumption and reduced production due to organ dysfunction, namely the dysfunction of the liver, where it is synthesised, is likely the reason why protein C levels become reduced in septic patients, contributing to the development of a procoagulant state and increased mortality.[203,220–223] Protein S, another glycoprotein which acts as a cofactor to aPC in the inactivation of FVa and FVIIIa, may also contribute to the development of a procoagulant state by becoming reduced in a similar fashion.[182,191,203,224] The anticoagulant capability of Protein S is not confined to its interaction with protein C, as it is also responsible for enhancing the interaction between TFPI and FXa and inhibiting the complex formed between FVa and FXa.[191,203,225] Moreover, endotoxin, IL-1, and TNFα are all responsible for inhibiting the expression of thrombomodulin and EPCR.[184,222,226–228] Thrombomodulin activity is further impaired by neutrophil elastase which cleaves it from the endothelial cell membrane, generating a less active form of the receptor.[184,185,222,228,229]

AT is another circulating glycoprotein with anticoagulant properties mainly due to its ability to bind to and inhibit thrombin, as well as other coagulation factors such as FIXa and FXa.[191,222,230–232] The presence of heparin highly improves the inhibitory ability of AT. However, physiological circulating levels of heparin are not high enough to significantly contribute to the activation of AT.[191,231,233] Thus, in the absence of heparin, AT is activated by endogenous glycosaminoglycans, such as heparan sulphate, expressed on the surface of endothelial cells.[224,232–235] In sepsis, AT levels are considerably reduced due to its consumption caused by continued thrombin generation. Reduced synthesis and degradation by neutrophil elastase also contribute to the depletion of AT during severe inflammation.[182,193,203,222,223] Furthermore, proinflammatory cytokines released during the inflammatory response suppress the production of glycosaminoglycans on the endothelial surface, subsequently impairing AT function.[184,193,224,235]

1.3.3. SUPPRESSION OF FIBRINOLYSIS

Fibrinolysis exists as a parallel mechanism through which haemostasis is regulated.[188,191,235,236] For fibrinolysis to occur, plasminogen must be converted to plasmin. Plasminogen is primarily synthesised in the liver and requires posterior activation to plasmin to perform its fibrinolytic function.[203,236,237] Plasminogen is usually activated once incorporated into the clot, which is only possible due to its affinity for fibrin.[203,238] Following its conversion, plasmin causes the proteolysis of fibrin, dissolving the fibrin clot into fibrin degradation products, which are cleared by the liver.[191,238] Fibrin itself enhances plasminogen activation.[190,239]

The main plasminogen activating enzymes include the tissue plasminogen activator (TPA) and the urokinase-type plasminogen activator (UPA).[188,203,236,239] TPA is the most important plasminogen activator. It is synthesised by endothelial cells and released both constitutively and as a response to a variety of triggers including cell injury and thrombin stimulation.[188,190,191,236,238,239] TPA requires the presence of fibrin to adequately activate plasminogen.[190,239,240] In comparison, UPA appears to play a minor role in the conversion of plasminogen to plasmin. It can, however, be produced by a larger number of cells, including monocytes, endothelial cells, and epithelial cells, and is released in response to cell activation by endotoxin and inflammatory cytokines.[241,242] Unlike TPA, UPA binds to specific cell surface receptors named urokinase-type plasminogen activator receptors (UPARs), and not fibrin, to activate plasminogen.[203,236,240]

Fibrinolysis is limited by the activity of the previously mentioned TAFI and by plasminogen activator inhibitors (PAIs), both of which are suppressed by aPC. While TAFI reduces the rate of fibrinolysis by protecting fibrin from the breakdown caused by plasmin, PAIs prevent the activation of plasminogen by irreversibly inhibiting both TPA and UPA.[191,203,204,235,243] The main PAI is the plasminogen activator inhibitor type 1 (PAI-1), which is produced by a miscellany of cells including platelets, leukocytes, and endothelial cells.[203,219,236,239] Fibrinolysis is further suppressed by circulating plasmin inhibitors, such as alpha-2-antiplasmin and alpha-2-macroglobulin.[191,203,219,238]

During sepsis, the elevated levels of TNFα and IL-1 in circulation cause an increased secretion of both TPA and UPA.[132,193] The resulting rise in fibrinolytic activity is rapidly counteracted by a sustained release of PAI-1, strongly inhibiting fibrinolysis and contributing to a procoagulant environment.[132,188,244]

1.3.4. ADDITIONAL INTERACTIONS BETWEEN INFLAMMATION AND COAGULATION

Systemic inflammation contributes to the development of a procoagulant state in septic patients. The opposite, however, is also true, as blood coagulation further stimulates the inflammatory response.[182,184] Procoagulant proteases such as TF, FVIIIa, FXa, and thrombin can activate protease-activated receptors (PARs), which are expressed by platelets, leukocytes, and epithelial and endothelial cells. PARs mediate cell activation, and thus, once activated themselves, these receptors can trigger the synthesis of inflammatory mediators that further enhance the inflammatory response.[207,245–247]

The inhibition of physiological anticoagulant and fibrinolytic mechanisms further contributes to the progression of a proinflammatory environment since a large number of the anticoagulant enzymes involved in these processes also hold anti-inflammatory properties.[184,243,247] For example, both aPC and TFPI appear to inhibit leukocyte activation and cytokine production. [184,210,248,249]

In addition to the presence of invading microorganisms and tissue damage, blood coagulation is also involved in the complex process that is the activation of the complement system. While initially helpful in the elimination of spreading microorganisms, the sustained activation of the complement system is, as previously mentioned, detrimental, by inducing further proinflammatory activity and thus contributing to the development of MODS.[96,250] The activation of the complement system amplifies coagulation by inducing platelet activation and stimulating TF and PAI-1 expression.[251–253] Additionally, the complement system also inhibits the anticoagulant activity of protein S.[225,254] In turn, thrombin can activate the complement system by cleaving two of its main proteins, complement component 3 (C3) and complement

component 5 (C5), into their activated form. Various other factors involved in the haemostatic process, including FXa, FXIIa, and plasmin, can also cleave and activate C3. The activation of these two complement components, however, can be suppressed by TAFI.[77,253,255]

The involvement of factor XII (FXII), also known as Hageman factor, in the development of dysfunctional coagulation in sepsis is not fully understood. FXII was part of the old cascade model of the coagulation process as one of the initial factors of the intrinsic pathway.[191,256,257] While the role of FXII in sepsis-induced coagulopathy appears to be secondary and controversial, it does seem that bacteria are capable of directly activating it.[83,256–259] Interestingly, the activated form of this glycoprotein can activate the complement system by cleaving complement component 1 (C1), and the inhibition of this activation appears to reduce complement activity.[77,257]

1.3.5. DISSEMINATED INTRAVASCULAR COAGULATION (DIC) IN SEPSIS

While blood coagulation may start off as a beneficial process that allows the entrapment of bacteria and healing of wounds, it quickly becomes extremely harmful once excessively activated.[132,204,260] DIC is the result of the combination of the previously described haemostatic abnormalities that occur during sepsis. Severe trauma is another condition amongst critically ill patients which frequently develops an exaggerated inflammatory response that results in DIC, mostly due to the massive exposure of damaged tissue to the blood circulation.[183,261,262] Trauma-induced DIC should not be confused with acute traumatic coagulopathy (ATC) which is a possible consequence of acute trauma, associated with increased fibrinolysis. The process of ATC development appears to be controversial and yet to be entirely understood.[262–264]

The process through which coagulopathy occurs and causes organ dysfunction is nearly identical in both infectious and non-infectious causes of SIRS, and some previous studies

have even shown no significant differences in systemic cytokine patterns, platelet function, and clot formation amongst patients with sepsis and nonseptic SIRS.[249,265,266]

Early DIC is associated with an hypercoagulable and prothrombotic state. As the condition progresses, however, a shift occurs towards hypocoagulability and haemorrhage. Ultimately, patients with DIC end up manifesting both widespread microvascular thrombosis and diffuse bleeding as a result of the continuous consumption and subsequent depletion of platelets and coagulation proteins, caused by the incessant activation of the coagulation system.[132,265] While haemorrhage may lead to the development of anaemia and further loss of platelets and coagulation factors, thrombosis remains one of the main mechanisms leading to organ dysfunction.[137,183,267,268] Hepatic dysfunction can further aggravate the occurring coagulopathy since the majority of coagulation factors are synthesised in the liver.[137,191,269]

1.3.6. ABNORMAL COAGULATION TIMES IN SEPSIS

The occurrence of coagulopathy in sepsis reproduces abnormal results when assessing coagulation function. The activated partial thromboplastin time (aPTT) test and the prothrombin time (PT) test are two of the most commonly used screening tests for coagulation abnormalities, including the ones observed in DIC.[199,249,270] These tests are based on the evaluation of the integrity of the extrinsic and intrinsic pathways of the coagulation process, according to its cascade model. PT evaluates the integrity of the extrinsic pathway while aPTT verifies the state of its intrinsic counterpart. Both of these tests are also affected by abnormalities in the final common pathway.[199,270] Despite the current cell-based model description of the coagulation system, these tests can be used to estimate the concentration of the different coagulation factors.[271] The tests do not, however, indicate the cause of coagulation factor depletion.[270]

The aPTT test represents the time it takes for a fibrin clot to be formed upon FXII activation, expressed in seconds.[272] It evaluates deficiencies of the following factors: prothrombin, fibrinogen, FV, FVIII, FIX, FX, FXII, and factor XI (FXI), which is also known as plasma thromboplastin antecedent.[261,270,272] The test consists of adding a

phospholipid platelet substitute, a FXII activator, and calcium to a plasma sample, generating the activation of the coagulation system.[270,272] Similarly to the aPTT test, PT measures the time it takes for a clot to be formed in a plasma sample, following the addition of calcium and phospholipids with tissue factor.[270,273] The PT test detects deficiencies of the following factors: prothrombin, fibrinogen, FV, FVII, and FX.[261,270,273] Both tests can be performed manually or with the use of automated devices.[272,273]

Despite their utility, these tests are accompanied by some limitations. Variables such as prolonged storage, sample contamination, and inadequate sample volume all affect test results. Additionally, aPTT and PT tests do not provide information in regards to platelet function or fibrin clot stability. Furthermore, the reference values and sensitivity of aPTT and PT tests depend on the instrument and reagents used to perform the tests themselves.[270,271,273]

The coagulation factor consumption observed in sepsis-induced DIC may lead to the prolongation of both aPTT and PT.[261,271–273] The absence of abnormal results in any of these tests should not be enough reason to discard the presence of sepsis, since the prolongation of coagulation times may not be observed in all affected patients. However, it appears to be likely for septic patients to exhibit an elevated result in at least one of the two tests.[140,183,261,274,275] For example, in their study regarding organ dysfunction in dogs with sepsis, Kenney *et al.*[138] considered the presence of coagulation dysfunction once an increase of at least 25%, in either aPTT or PT, was observed. In this same study, 60.5% of the septic dog population met the criteria. In a different study by de Laforcade *et al.*[57] on haemostatic changes in dogs with naturally occurring sepsis, the same standards regarding aPTT and PT were used to identify DIC. In this study, however, only 25% of septic dogs fulfilled the criteria.

Additionally, coagulation time prolongation may occur before clinical signs of inadequate haemostasis become apparent.[271] Shortened coagulation times are, for the most part, considered to be of limited clinical significance.[272,273]

1.4. RECOMMENDED SCORING SYSTEMS FOR THE ASSESSMENT OF ORGAN DYSFUNCTION IN SEPSIS

Many human organ function scoring systems have been developed to evaluate critically ill patients and guide therapy. However, a veterinary-specific MODS scoring system is yet to be created, and the application of human organ failure scores in veterinary patients is still subject of research.[84,276]

As previously mentioned, the authors of Sepsis-3 proposed the use of the SOFA score for the assessment of organ dysfunction in septic patients.[24] The SOFA score was created in 1994 and is based on the evaluation of six organ systems: respiratory, hematologic, hepatic, cardiovascular, neurologic, and renal.[277] SOFA was initially an acronym for "sepsis-related organ failure assessment" score but was later renamed due to its applicability to nonseptic patients.[276] Each organ system is given a score, from zero to four points, according to how altered its function is. Mortality rates are expected to rise in correlation with the increase in score for each organ system. All six scores are then combined to generate the total SOFA score, which ranges from zero to 24 points.[277,278] The authors of Sepsis-3 considered a total SOFA score equal to or greater than two points to be representative of organ dysfunction in septic patients and reflective of an increased mortality risk of approximately 10%.[24,26]

Even though the SOFA scoring system was created to be used in human patients, a study by Ripanti *et al.*[279] with the goal of testing the applicability of the SOFA score in the assessment of outcome in critically ill dogs has shown promising results that support its use in such canine patients. This same study used slightly different values from the ones initially proposed for the criteria utilised by the SOFA scoring system when applied to human patients. Some authors have also advocated the use of the modified version of the Glasgow Coma Scale in the evaluation of neurologic function in veterinary patients, instead of its original version (Appendix A, p. ii).[84,280–282] The adaptation of the SOFA score criteria to veterinary patients can be observed in Table 2.

Table 2. The sequential organ failure assessment (SOFA) score criteria for veterinary patients (adapted from references 84 and 279).

System	Score				
	0	1	2	3	4
Respiratory PaO_2/FiO_2 (mmHg)[A]	> 400	< 400	< 300	< 200 (Ventilated)	< 100 (Ventilated)
Hematologic Platelets ($10^3/mm^3$)	≥ 150	≤ 150	≤ 100	≤ 50	≤ 20
Hepatic Bilirubin (mg/dl)	< 0.6	0.6 – 1.4	1.5 – 5.0	5.1 – 11.0	> 11.1
Cardiovascular MAP [B] (mmHg) or vasopressors [C]	≥ 60	< 60	Dopamine < 5 or dobutamine (any dose) [C]	Dopamine > 5 or epinephrine ≤ 0.1 or norepinephrine ≤ 1 [C]	Dopamine > 15 or epinephrine > 0.1 or norepinephrine > 1 [C]
Neurologic Modified Glasgow Coma Scale	> 14	13-14	10-12	6-9	<6
Renal Creatinine (mg/dl)	< 1.4	1.4 – 1.9	2.0 – 3.4	3.5 – 4.9	>5

[A] PaO_2/FiO_2, Partial pressure of oxygen/fraction of inspired oxygen. [B] MAP, Mean arterial pressure. [C] Vasopressors are administered for a minimum of one hour. Doses given are in µg/kg/min.

The SOFA scoring system is incredibly helpful in the identification of organ dysfunction, but it may become too time-consuming and impractical to apply, both outside the ICU and in smaller and less equipped veterinary centres, due to its complexity and the need for laboratory testing. Even though it may be performed retrospectively using criteria assessed in the past as part of routine screening, the SOFA score does not represent a tool of speedy results, which led to the creation of its simplified version: the qSOFA score.[24–26,36]

The qSOFA score is a simple tool that allows the identification of patients with suspected infection, outside the ICU, who are at a higher risk of developing a poor outcome.[24] The qSOFA score incorporates three criteria: increased respiratory rate, altered mentation, and low systolic blood pressure (Table 3). One point is awarded for the fulfilment of each criterion, up to a maximum of three points. A score of two or more points is considered to be predictive of prolonged ICU stay and in-hospital death and should prompt further examining.[24,26,36]

Table 3. The quick sequential organ failure assessment (qSOFA) score criteria (adapted from reference 24).

qSOFA Criteria
Respiratory rate ≥ 22 breaths/min
Modified Glasgow Coma Scale score < 15
Systolic blood pressure ≤ 100 mmHg

The qSOFA scoring system should not be used as a diagnostic tool for sepsis but rather as an early warning system that encourages clinicians to further evaluate patients with suspected infection for the presence of organ dysfunction, to initiate or adapt therapy, to increase the frequency of monitoring, and to consider a transfer to an ICU.[24,36] Patients without suspected infection who display signs of qSOFA scores equal to or greater than two points should also be targets of increased surveillance and concern. Likewise, patients who are very likely to be infected despite having lower qSOFA scores should not be disregarded.[36,40]

The predictive validity of the qSOFA score appears to be similar to that of the SOFA score outside the ICU. In an ICU setting, however, SOFA possesses greater prognostic accuracy, likely due to the influence of ongoing organ support through mechanical ventilation and vasopressor therapy.[26,27]

Although a tool of great utility, the qSOFA score is not flawless. It is possible for septic patients to score less than two points since other forms of organ dysfunction such as hematologic, renal, and hepatic dysfunction are not being evaluated when qSOFA is applied. The opposite may also occur when nonseptic patients are given high qSOFA scores even though the degree of their tachypnea, altered mental status or hypotension is not high enough to meet the SOFA criteria evaluated afterwards.[36] Furthermore, it is possible that patients do not manifest the qSOFA criteria at all until late in the disease process when it might be too late for initiating treatment.[29,40] Thus, organ function should not be evaluated exclusively through the use of scoring systems as these are not perfect. Clinical judgement and further testing should be applied if the clinician is still suspicious of the presence of sepsis even after examining patients with low SOFA or qSOFA scores (Figure 5).[36,283]

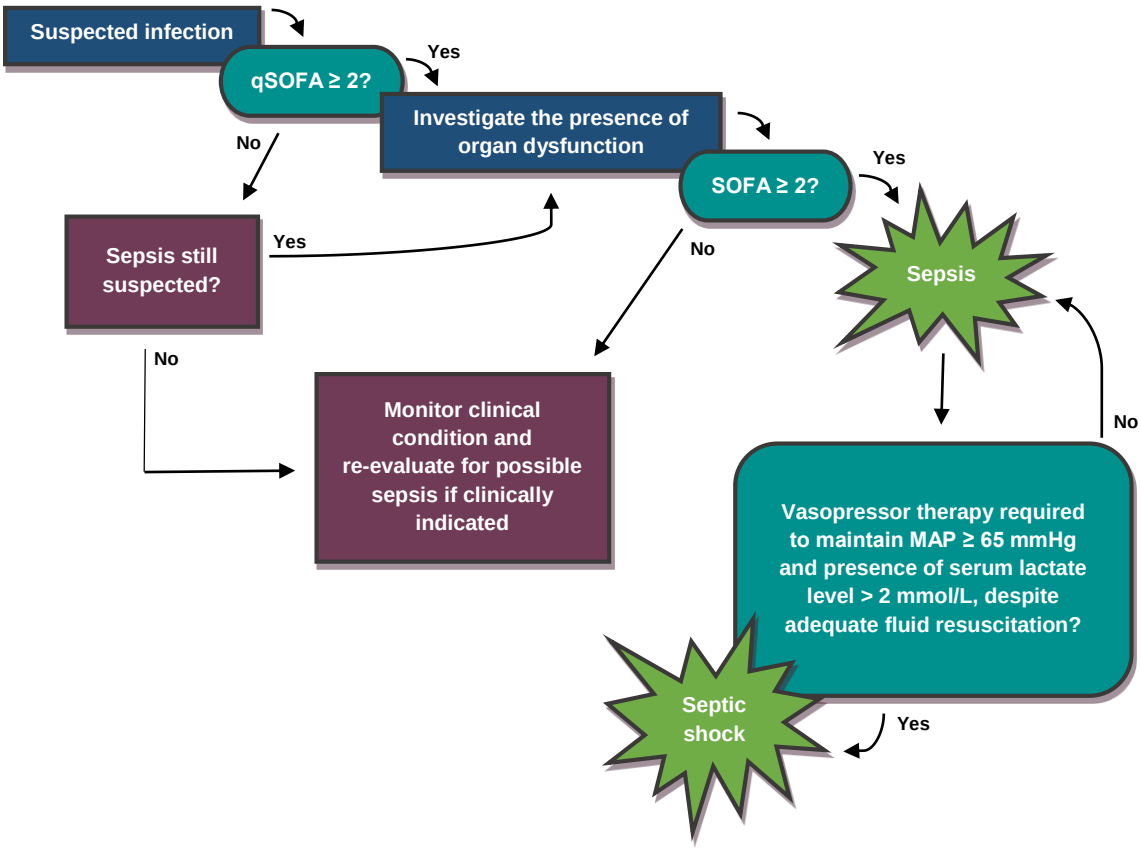

Figure 5. The identification process for sepsis and septic shock according to Sepsis-3 (adapted from reference 24).

2. STUDY – THE PROGNOSTIC VALUE OF ABNORMAL COAGULATION TIMES

2.1. STUDY INTRODUCTION

Coagulation dysfunction seems to be a common occurrence in critically ill patients with sepsis or at risk of entering a septic state. The analysis of how this dysfunction evolves and correlates to disease and injury severity could lead the way into creating an additional tool of prognosis for the critically ill veterinary patient. Additionally, the qSOFA scoring system can be very useful in veterinary medicine since it can be quickly applied to our patients. However, like most tools at our disposal, qSOFA is not perfect. Thus, the main idea behind this study was not only to test the effectiveness of coagulation markers in the prediction of outcome but also to analyse if they could serve as a tool to strengthen the information given to the clinician by the qSOFA score.

2.2. OBJECTIVES

The aim of this study was to investigate the use of aPTT and PT, at admission to the ICU, as biological markers of coagulation dysregulation in critically ill patients who are at risk of developing sepsis, as well as their correlation with disease severity and outcome. A secondary objective was to correlate aPTT and PT with the qSOFA scoring system at the moment of admission to the ICU and evaluate their combined use as a tool of prognostic value amongst critically ill patients.

2.3. MATERIALS AND METHODS

2.3.1. STUDY POPULATION

A total of 43 dogs were enrolled in the study between September 2016 and March 2017. Breeds included 17 mixed breeds, four Labrador Retrievers, two Bullmastiffs, two German Shepherds, two Pekingese, two Yorkshire Terriers, and one of each of the following: Beagle, Belgian Shepherd, Boxer, Bull Terrier, Chihuahua, Czechoslovakian Wolfdog, Dachshund, English Cocker Spaniel, German Shorthaired Pointer, Great Dane, Miniature Pinscher, Miniature Poodle, Pomeranian, and Portuguese Sheepdog. Twenty-six of the patients were male (60.5%), and 17 were female (39.5%). The median age was six years (range, 0.2-16 years). Appendix B (see p. iii-vi) incorporates a table with all the collected information regarding the dog population enrolled in this study.

2.3.2. STUDY DESIGN

This study was conducted in a veterinary hospital setting. Every dog that was presented for consultation during the six-month period was evaluated for the presence of SIRS, sepsis or coagulopathy. Inclusion criteria were the presence of clinical signs of bleeding, coagulopathy, infection, shock or SIRS. Patients with polytrauma, organ dysfunction or neoplasia were also included. Dogs that died before blood collection or whose owners did not consent to this procedure were excluded from the study. Other species were also excluded.

Variables recorded for each subject included: signalment (breed, age and sex), diagnosis, aPTT, PT, qSOFA score, and duration of hospitalisation and post-discharge treatment. Coagulation testing and qSOFA scoring were performed at the moment of admission to the ICU and continued to be applied and monitored throughout hospitalisation. All patients received specific treatment directed at their condition during hospitalisation. Those who survived hospitalisation continued receiving treatment at home administered by their owners and were re-evaluated on a weekly basis.

2.3.3. BLOOD SAMPLING AND COAGULATION TESTING

Samples were collected for aPTT and PT determination from all dogs by atraumatic jugular venepuncture with sterile disposable syringes and needles (2 ml syringes and 20 gauge needles). The puncture site was previously shaved and aseptically prepared with chlorhexidine gluconate 2% topical solution. A study by Bauer *et al.*[284] suggests that different blood sample collection techniques do not alter coagulation testing results in dogs. A drop of the collected blood was then applied to a test strip (qLabs® Vet Coag Panel 2 Test Strip, Micropoint Bioscience inc., Santa Clara, USA) connected to a portable aPTT and PT analyser (qLabs® Vet Coag Panel 2 PT/APTT Combo, Micropoint Bioscience inc., Santa Clara, USA) (Figure 6). The portable device can detect the test strips on insertion and heat them up to a pre-set operating temperature while capillary channels transfer the blood to reaction zones to coagulate. The meter then detects the changes in these reaction areas and identifies a clot endpoint. These clot endpoints for both aPTT and PT testing are then converted to values which are more familiar to the clinician. The reference intervals of aPTT and PT for healthy dogs using the qLabs® test, stated by the manufacturer, are the following: an aPTT of 75 to 105 seconds and a PT of 14 to 19 seconds.

Figure 6. qLabs® Vet Coag Panel 2 device and test strips (original figure).

2.3.4. STATISTICAL ANALYSIS

Excel software (Microsoft Corporation, Redmond, Washington, USA) was used for data management, and SPSS software (IBM, Armonk, New York, USA) was used to analyse data statistically. Chi-square tests were used to test independence between two categorical variables. T-tests were conducted to evaluate differences between two continuous variables. One-way analysis of variance (ANOVA) tests were performed to determine the existence of any differences between the means of more than two unrelated groups. Tukey's honestly significant difference (HSD) tests were used to follow-up statistically significant ANOVA results. Pearson product-moment correlation coefficient tests were conducted to measure the association between two variables. For this statistical analysis, each qSOFA score (0, 1, 2, and 3) was considered a categorical variable. All relevant or statistically significant relations resulting from this analysis can be found in the following results.

2.4. RESULTS

2.4.1. DIAGNOSIS

Fifteen of the 43 subjects died during treatment, which represented an overall mortality rate of 34.9%. Two dogs were euthanised due to the deterioration of their clinical condition, and the remaining 13 died spontaneously. Underlying causes of disease or injury were diagnosed on admission, such as trauma (n=10), neoplasia (n=9), gastrointestinal disease (n=8), toxicological emergency (n=4), urinary tract disease (n=4), infectious disease (n=3), neurological disorder (n=2), autoimmune disease (n=1), prostate disease (n=1), and respiratory disease (n=1). Figure 7 shows the survival and non-survival rates of each of these diagnoses. Neoplasia was the most common cause of death (33.3%), followed by trauma (26.7%), and urinary tract disease (13.3%). Despite a large number of subjects diagnosed with a gastrointestinal disease, only one of them succumbed to its illness.

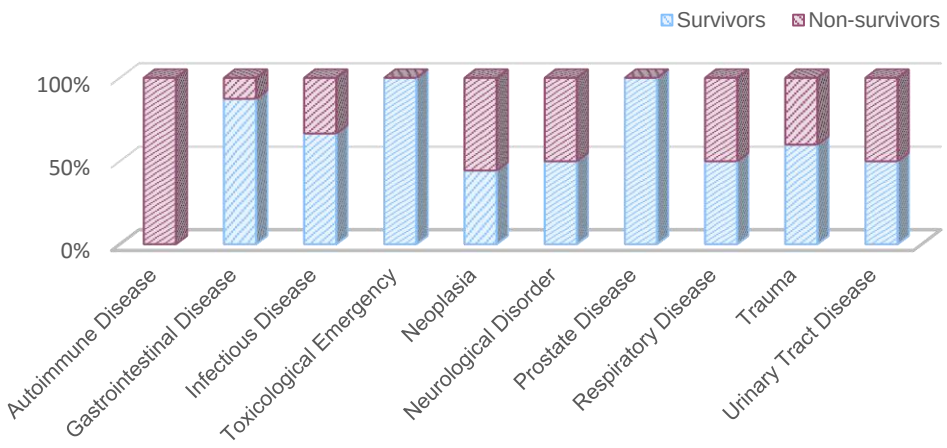

Figure 7. Bar graph representing survival and non-survival rates of each of the diagnosed underlying causes of illness or injury.

2.4.2. BREED, AGE, AND SEX

The patients were divided into two groups in order for the relationship between their breed and outcome to be analysed: purebred and mixed breed patients. Six out of 17 mixed breed patients died during treatment which represents a mortality rate of 35.3% for this group. A nearly identical mortality rate of 35% was seen amongst purebred patients, having nine out of 26 patients died during treatment. No statistically significant relationship was found between breed and outcome.

The average patient age in this study was 6.3 years. Survivors had a mean age of 5.7 years and non-survivors had a mean age of 7.5 years. No statistically significant relationship was found between age and outcome, but the results suggest that survivors were more inclined to be younger than non-surviving patients.

A chi-square test of independence was performed to examine the association between sex and mortality, and no significant relationship between these two variables was found. However, females had a noticeably higher mortality rate (41.2%) than males (30.8%). Figure 8 shows the number of survivors and non-survivors of each sex.

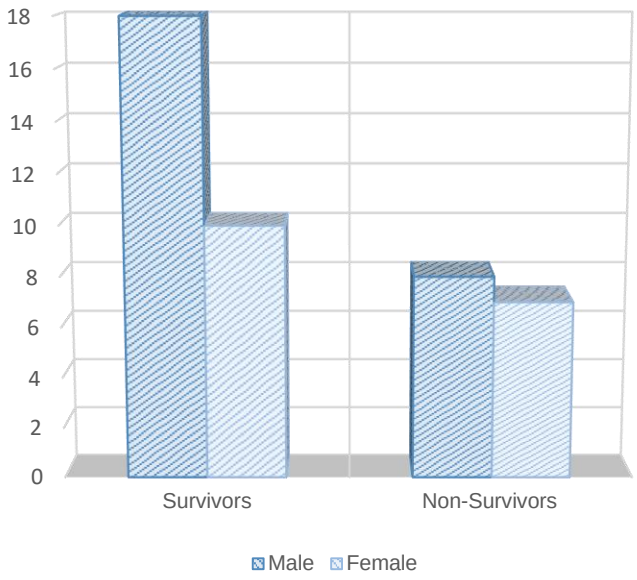

Figure 8. Bar graph representing the number of survivors and non-survivors of each sex.

2.4.3. qSOFA, ACTIVATED PARTIAL THROMBOPLASTIN TIME, AND PROTHROMBIN TIME

The qSOFA scoring system was applied on admission to identify 10 patients with a score of zero points, 13 patients with a score of one point, 17 patients with a score of two points, and three patients with a score of three points. Eight of the 15 (53.3%) dogs that died during treatment scored two points on admission. Dogs with a score of three points had the highest mortality rate (66.7%). Table 4 shows the mortality rate associated with each score. A chi-square test of independence was performed once more, and no significant relation was found between qSOFA scores and mortality. However, the number of non-survivors increased as qSOFA scores got higher, despite a similar number of survivors associated with each of the first three scores.

Table 4. The mortality rate associated with each quick sequential organ failure assessment (qSOFA) score in the present study.

qSOFA score	No. of patients	No. of survivors	No. of non-survivors	Mortality
0	10	9	1	10%
1	13	9	4	30.8%
2	17	9	8	47.1%
3	3	1	2	66.7%

A one-way ANOVA test was conducted to compare the effect of qSOFA scores on aPTT levels. There was a statistically significant difference between groups [$F(3,39) = 3.420$, $p = 0.026$]. A Tukey post hoc test revealed that aPTT values were significantly higher in patients with a qSOFA score of 2 points (121.5 ± 14.1, $p = 0.029$) compared to patients with a qSOFA score of 1 point (110.4 ± 7.9). There was no statistically significant difference between the other qSOFA scores. Mean aPTT values associated with each qSOFA score can be observed in Figure 9. A similar analysis was made for PT levels, but no statistically significant results were found.

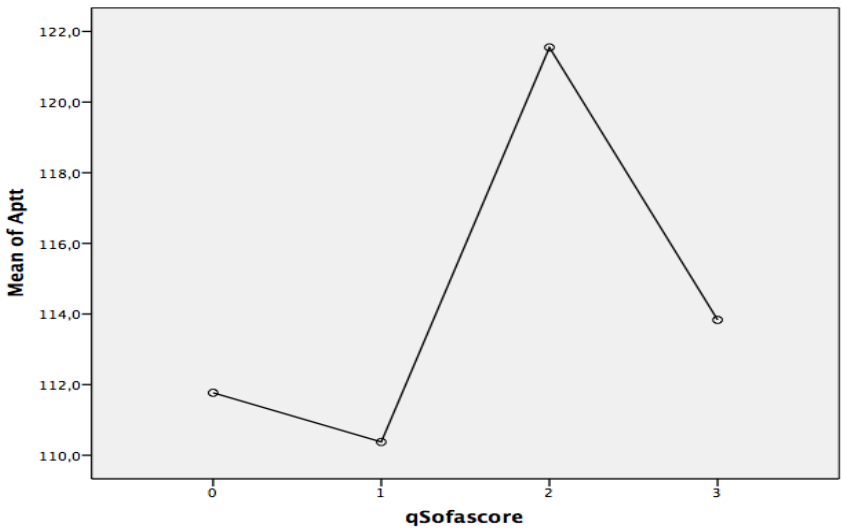

Figure 9. Graphical representation of mean activated partial thromboplastin time (aPTT) values associated with each quick sequential organ failure assessment (qSOFA) score.

A Pearson product-moment correlation test was run to determine the relation between aPTT and PT. There was a positive correlation between aPTT and PT which was statistically significant (r = 0.406, n = 43, p = 0.005). Figure 10 shows this correlation.

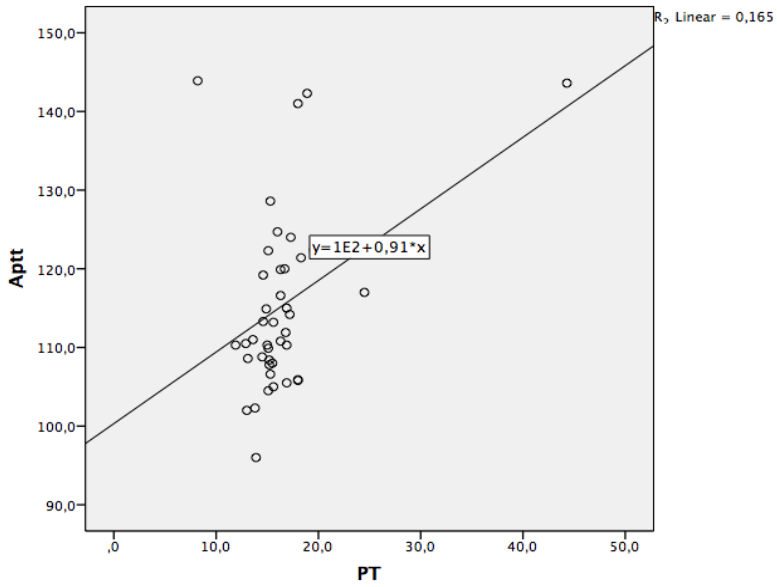

Figure 10. Scatter graph representing the positive correlation between activated partial thromboplastin time (aPTT) and prothrombin time (PT).

Regarding outcome, mean values for aPTT and PT were very similar between surviving and non-surviving patients. Surviving patients presented a mean aPTT value of 115.0 ± 11.7 seconds and PT value of 16.8 ± 5.8 seconds. Non-survivors showed a mean aPTT value of 116.0 ± 10.7 seconds and PT value of 16.1 ± 3.5 seconds.

2.4.4. LENGTH OF TREATMENT AND OUTCOME

Ten of the 15 (66.7%) patients that died did so between their first and fifth day of hospitalisation and overall treatment. The other five deaths occurred while the patients were being treated at home, after having survived hospitalisation. Three of these five deaths (60%) were caused by neoplastic disease. The mean number of days of

hospitalisation and treatment associated with each outcome is represented in Table 5, which demonstrates how most deaths occurred early in treatment.

Table 5. Mean length of hospitalisation and treatment of survivors and non-survivors.

	Mean length of hospital stay (days)	Mean length of post-discharge treatment (days)	Total mean length of treatment (days)
Survivors	6.39 ± 7.20	19.00 ± 17.57	25.40 ± 19.08
Non-survivors	5.47 ± 6.42	10.20 ± 20.84	15.67 ± 22.50

2.5. DISCUSSION

The results of this study show a shortage of statistically significant relationships between the examined variables. However, there are a few statistically relevant relationships and other interesting findings that are worth considering.

Based on the analysis of the different diagnoses made on admission, it is evident that trauma and neoplasia represented the most common reasons for hospitalisation as well as the leading causes of death. These are also amongst the most common causes of SIRS found in veterinary literature.[44] It is important to mention that the diagnoses made in this study were secondary to its main objective and that patients were included by simply meeting the inclusion criteria. Therefore, it is possible that different underlying causes of hospitalisation were partly or entirely responsible for fluctuations in aPTT and PT values and outcome, regardless of qSOFA score or septic status. It is understandable that higher mortality rates in cases of neoplasia or more exuberant coagulation dysfunction in toxicological emergencies, such as the ingestion of anticoagulant rodenticides (which is the case of patient number seven, who presented the highest aPTT and PT values on admission), are clear examples of how disease pathophysiology can influence these results.[285] The fact that very distinct aetiologies were included together, while each being represented by a small number of subjects, was also the reason why it was impossible to

search for any statistically significant relationships between diagnosis and other variables. The inclusion of patients with very diverse diagnoses is an evident flaw in this study, and it would be interesting for future studies on this subject to proceed with similar investigations for each particular disease or injury.

It was interesting to find that both purebred and mixed breed patients had very similar mortality rates. However, each breed had a very minimal number of representatives, so additional studies with more subjects would be required for any conclusions to be drawn regarding breed-related susceptibility to sepsis. A study by Nemzek *et al.*[286] suggests that breed affects cytokine production in dogs and consequently influences the host response to infection. Thus, more studies targeting this breed-related response would be interesting to develop.

Even though no statistically significant relations were found in the analysis of patient age, the results suggest that older patients might be more affected by sepsis than younger ones. The results of a study performed by Antonelli *et al.*[287] in human trauma patients also revealed that survivors were much younger than non-survivors. Additionally, a human medicine study carried out by Starr *et al.*[288] showed that the adipose tissue of aged mice had an increased inflammatory potential when compared to that of young mice, which suggests that adipose tissue might be involved in the mortality of older patients. In another human medicine study by Walsh *et al.*[289] regarding the outcome of critically ill patients with increased prothrombin time, it was found that patients with prolonged PT were not only more likely to be older but also to be female and suffering from sepsis. Regarding the impact of sex in this study, females displayed a higher mortality rate than males, but there was no statistically significant relationship between sex and outcome amongst the results. It was suggested in a human medicine study by Schreiber *et al.*[290] that hypercoagulability following trauma is more common amongst females and is associated with increased mortality. Interestingly, three out of four females (75%) died following trauma in this study, whereas only one out of six males (16.7%) died in the same circumstances.

The qSOFA scoring system was not only applied in this study because of its quick and straightforward nature but also because an aim of this study was to test if combining qSOFA with coagulation markers would be beneficial in the prediction of mortality in

critically ill patients, similarly to what has been investigated before in human and veterinary patients regarding plasma lactate concentration.[291,292] The obtained results concerning qSOFA scores show that the majority of patients who died were scored with two points. Interestingly, the number of surviving subjects with a score of zero, one, and two points was the same, yet more deaths were observed as these scores increased. Patients with a score of three points had the highest mortality rate and were part of the only score with fewer survivors than non-survivors. However, it should be taken into consideration that this particular score was only represented by three subjects, which certainly affects the reliability of these results. Regardless, these results suggest that higher qSOFA scores tend to be associated with increased mortality rates. Therefore, the results of the present study are not only in agreement with the authors of the latest sepsis definition but also with a variety of human studies that revealed an increase in mortality as more qSOFA criteria were met.[24,26,27,292–295]

A statistically significant relationship was found between qSOFA scores and aPTT values, which suggests that patients with a qSOFA score of two points tend to have higher aPTT values than the ones with lower scores. These results seem to insinuate that coagulation dysfunction gets considerably more severe as patients progress from a qSOFA score of one point to a score of two points, which makes sense given how mortality also appears to increase in patients with a score of two or more points, as previously mentioned. It would have been beneficial for this study to have additional patients with a qSOFA score of three points, not only to tackle the issue presented previously regarding mortality amongst patients with such score but also to properly analyse if these patients would have higher aPTT values than the ones with a score of two points.

A study on haemostatic changes in dogs with naturally occurring sepsis by Laforcade *et al.*[57] showed that dogs with sepsis tend to have higher aPTT and PT values when compared to controls, within 24 hours of admission, which is in line with the results of the present study if we consider a qSOFA score of two points to be highly indicative of sepsis. A different study by Ok *et al.*[296] also reported an increase in aPTT and PT in dogs with sepsis when compared to healthy dogs. Contrary to what was seen regarding aPTT values, no relationship was found between qSOFA scores and PT values. These findings

seem to suggest the possibility of a relationship between aPTT values at admission and mortality. These results are also suggestive of a weaker or non-existent link between PT values at admission and mortality, which would be in agreement with other studies that have found only prolonged aPTT to be indicative of outcome. A previous study by Holowaychuk *et al.*[297] showed that increased aPTT at hospital admission in dogs with severe traumatic injuries was correlated with injury severity and was also predictive of mortality. In this same study, however, PT was not predictive of outcome. A retrospective study by Bentley *et al.*[60] had similar results by analysing two populations of dogs with septic peritonitis. In this study, aPTT was significantly prolonged amongst non-survivors, but PT was not significantly altered. Shipov *et al.*[298] also found increased aPTT to be highly indicative of worse outcomes in cases of canine monocytic ehrlichiosis. Even though PT was prolonged in some dogs enrolled in this study, only aPTT was significantly prolonged upon presentation amongst non-survivors. Additionally, a study by Dengate *et al.*[299] revealed the presence of increased aPTT, but not PT, in dogs with thrombosis secondary to underlying illnesses, when compared to healthy controls.

The correlation between aPTT and PT was tested, and it does seem that when one increases, the other tends to do so as well. Despite the previous results, no statistically significant relationship was found between isolated aPTT or PT values and outcome, and both survivors and non-survivors had similar mean results for these two variables, which might indicate that aPTT and PT levels by themselves might not be the best tools for the prediction of outcome. These results are similar to those of a study by Bentley *et al.*[300], which concluded that aPTT and PT were not useful in predicting mortality in dogs with septic peritonitis. In this same study, however, aPTT was still higher amongst non-survivors, which is in line with what was previously mentioned. Adamantos *et al.*[301] also found that abnormal aPTT and PT values were not correlated with outcome in a population of dogs who showed signs of bleeding and were infected with *Angiostrongylus vasorum.*

Other authors, however, have had distinct results regarding coagulopathy at admission and outcome. In a study by Dhainaut *et al.*[302], the results indicated that the presence of coagulation abnormalities in human patients during the first day of sepsis was predictive of either new organ dysfunction or progression and delayed resolution of already existing

organ dysfunction and consequently increased mortality rate. In a retrospective human study by Benediktsson *et al.*[303], ICU admission values of aPTT and PT in patients with severe sepsis or septic shock were associated with outcome. In a study regarding cats with DIC by Estrin et al.[304], the authors observed that aPTT was prolonged in all the cats affected by the disease. In this same study, PT was also prolonged in a significant number of cats with DIC, and the median PT of the non-surviving cats was significantly more prolonged than that of those who survived the disease. A study regarding heat stroke in dogs by Bruchim *et al.*[305] found early changes in PT and aPTT to be significantly associated with mortality. Likewise, a distinct study on the same subject of heat stroke by Bruchim et al.[306] also revealed the presence of longer aPTT and PT amongst non-surviving dogs, in comparison to those who survived the illness. A study by Gottlieb *et al.*[307], regarding trauma in dogs and cats, revealed that prolongation of both PT and aPTT was significantly related to injury severity in dogs. Other human studies concerning the subject of trauma also reported higher mortality rates amongst patients suffering from coagulopathy.[308,309] In a human study by Adamik *et al.*[310], the presence of coagulation disorders in septic patients, identified by thromboelastometry, was associated with higher mortality rates and increased endotoxin activity. Curiously, a study by Bauer & Moritz[311] showed that prolonged coagulation times and severe coagulopathy could be found in critically ill dogs, despite the presence of SIRS.

Finally, results showed that most deaths occurred between the first and fifth day of treatment and that after that period the majority of patients survived. This information suggests that the first five days of treatment are the most complicated and decisive in critically ill patients, and allows the clinician to properly inform and guide the owners of patients going through similar circumstances, regarding their hospitalisation. Similar results in human patients with sepsis have been reported previously by Blanco *et al.*[312]. In this study, a quarter of the non-surviving patients died within the first 48 hours of admission to the ICU, and more than half died within the first week.

This study was performed in a student internship setting at a veterinary hospital. Thus, the budget for the study was limited, and some patients could not be included due to the lack of consent by some of the owners and due to missing information amongst patient files. Individual treatment was also not considered since patients were suffering from

various diseases and injuries that required different medical intervention. The lack of a treatment protocol due to the volatile nature of the practice and diversity of diagnoses could have also influenced the results of this study.

Once more, the author suggests that further studies regarding this subject should seek to investigate sepsis and coagulation dysfunction in patients with specific underlying diseases in a controlled environment and with the implementation of standardised treatment plans for each disease, as well as with a larger amount of subjects.

2.6. CONCLUSIONS

Sepsis is a severe and complex condition amongst critically ill patients which can lead to organ dysfunction and ultimately death if proper treatment is not applied in a timely fashion. Patients affected by this condition may also suffer from altered blood coagulation and are at risk of developing a disseminated intravascular coagulation state.

Conclusions regarding the prognostic ability of aPTT and PT appear to be conflicting amongst different studies. The results of this study suggest that the assessment of aPTT and PT by itself, at admission to the ICU, may not be the most reliable way to form conclusions about the prognosis of critically ill veterinary patients. Contrary to these results, a variety of previous studies have found prolonged coagulation times upon presentation to be associated with outcome in critically ill patients.

However, aPTT was significantly increased in patients with a qSOFA score of two points in the present study, which in turn is associated with increased mortality. These results may indicate that pairing coagulation time data, namely aPTT, with the qSOFA score, at the moment of admission to the ICU, might be a more reliable way to predict the outcome of critically ill patients. These results also suggest that even though the qSOFA score does not directly evaluate haemostatic dysfunction, it is likely for patients with a score of two points to be affected by some degree of coagulopathy.

This study also shows that the first five days of treatment are the most crucial amongst the critically ill. This information is extremely useful not only in the clinician’s decision-

making process but also as a way to supply the owners of critically ill patients with a risk time frame based on evidence. Based on these results, rash decisions regarding euthanasia should be reconsidered as most patients that survive the first five days of treatment tend to recover from their illnesses. Owners of critically ill patients should be given a time frame of at least five days of treatment for their dog, at admission to the ICU. Having such a survival based time frame to present to owners of critically ill patients represents an immensely helpful and practical tool to any veterinary clinician. Despite not being part of the proposed goals for this study, these achieved results regarding patient survival have been considered to be tremendously important by the author.

There is still much to know about sepsis, and tools for its early detection should be further developed. At the present moment, the qSOFA scoring system seems to be a practical tool to use in veterinary medicine to assess risk amongst critically ill patients, but it should not be used as a singular diagnostic method. The SOFA score continues to be the thorough version of this tool for the recognition of organ dysfunction and sepsis between patients inside the intensive care unit, but its complex nature and need for laboratory testing might render it impracticable in a significant number of veterinary medical facilities.

Further studies should be encouraged on this subject, both in human and veterinary medicine, so that in the future sepsis can be tackled more comfortably by clinicians in both fields.

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APPENDICES

APPENDIX A

Modified Glasgow Coma Scale. (Adapted from references 280 and 281)

Modified Glasgow Coma Scale (MGCS)	
	Score
Motor activity	
Normal gait, normal spinal reflexes	6
Hemiparesis, tetraparesis, or decerebrate activity	5
Recumbent, intermittent extensor rigidity	4
Recumbent, constant extensor rigidity	3
Recumbent, constant extensor rigidity with opisthotonos	2
Recumbent, hypotonia of muscles, depressed or absent spinal reflexes	1
Brain stem reflexes	
Normal pupillary light reflexes and physiological nystagmus	6
Slow pupillary light reflexes and normal to reduced physiological nystagmus	5
Bilateral unresponsive miosis with normal to reduced physiological nystagmus	4
Pinpoint pupils with reduced to absent physiological nystagmus	3
Unilateral, unresponsive mydriasis with reduced to absent physiological nystagmus	2
Bilateral, unresponsive mydriasis with reduced to absent physiological nystagmus	1
Level of consciousness	
Occasional periods of alertness and responsive to the environment	6
Depression or delirium, capable of responding but response may be inappropriate	5
Semicomatose, responsive to visual stimuli	4
Semicomatose, responsive to auditory stimuli	3
Semicomatose, responsive only to repeated noxious stimuli	2
Comatose, unresponsive to repeated noxious stimuli	1

Score category	MGCS score	Suggested prognosis
I	3 to 8	Grave
II	9 to 14	Guarded
III	15 to 18	Good

APPENDIX B

Dog population enrolled in the study.

Patient ID	*Breed*	*Age (y)*	*Sex*	*Diagnosis*	*qSOFA score*	*aPTT*	*PT*	*Length of hospital stay (d)*	*Length of post-discharge treatment (d)*	*Total length of treatment (d)*	*Outcome*
1	Labrador Retriever	1	Female	Gastrointestinal Disease	2	122,7	20,7	18	47	65	Survived
2	German Shepherd	0,25	Male	Gastrointestinal Disease	2	108,6	13,1	15	11	26	Survived
3	Mixed Breed	3	Male	Infectious Disease	0	119,9	16,3	35	0	35	Survived
4	Mixed Breed	16	Female	Neoplasia	0	116,6	16,3	5	0	5	Survived
5	Mixed Breed	6	Male	Gastrointestinal Disease	3	110,3	16,9	5	18	23	Survived
6	Mixed Breed	2	Male	Toxicological Emergency	2	110,3	15	3	5	8	Survived
7	Labrador Retriever	1	Male	Toxicological Emergency	2	143,6	44,3	8	73	81	Survived
8	German Shorthaired Pointer	0,2	Male	Toxicological Emergency	1	114,9	14,9	3	0	3	Survived
9	Bullmastiff	2	Male	Gastrointestinal Disease	2	128,6	15,3	10	36	46	Survived
10	Mixed Breed	6	Male	Trauma	1	115	16,9	6	24	30	Survived

11	Chihuahua	5	Female	Neurological Disorder	2	122,4	19,5	8	19	27	Survived
12	Mixed Breed	1	Female	Trauma	1	107,8	15,2	2	0	2	Survived
13	Pomeranian	10	Male	Neoplasia	0	119,2	14,6	1	8	9	Survived
14	Mixed Breed	9	Male	Prostate Disease	0	111	13,6	1	38	39	Survived
15	Belgian Shepherd	6	Male	Trauma	0	108,4	15,2	4	10	14	Survived
16	Boxer	9	Female	Infectious Disease	0	102,3	13,8	3	2	5	Survived
17	English Cocker Spaniel	9	Female	Neoplasia	0	110,3	11,9	2	10	12	Survived
18	Yorkshire Terrier	3	Male	Trauma	2	142,3	18,9	4	38	42	Survived
19	Yorkshire Terrier	15	Male	Trauma	1	96	13,9	2	12	14	Survived
20	Mixed Breed	5	Female	Gastrointestinal Disease	1	113,3	14,6	1	34	35	Survived
21	Mixed Breed	5	Female	Gastrointestinal Disease	1	106,6	15,3	1	23	24	Survived
22	Mixed Breed	6	Male	Trauma	1	109,9	15,1	3	7	10	Survived

23	Mixed Breed	15	Male	Neoplasia	0	108,8	14,5	10	0	10	Survived
24	Portuguese Sheepdog	8	Female	Gastrointestinal Disease	2	105,8	18	7	19	26	Survived
25	Beagle	4	Male	Urinary Tract Disease	0	108	15,5	3	7	10	Survived
26	Labrador Retriever	2	Male	Respiratory Disease	1	111,9	16,8	14	30	44	Survived
27	Pekingese	3	Female	Toxicological Emergency	1	105	15,6	1	31	32	Survived
28	Dachshund	6	Male	Urinary Tract Disease	2	141	18	4	30	34	Survived
29	Labrador Retriever	13	Male	Neoplasia	3	114,2	17,2	9	9	18	Died
30	Czechoslovakian Wolfdog	1	Male	Gastrointestinal Disease	1	105,5	16,9	8	46	54	Died
31	Mixed Breed	11	Female	Infectious Disease	2	121,4	18,3	21	9	30	Died
32	German Shepherd	6	Male	Urinary Tract Disease	1	124,7	16	4	0	4	Died
33	Mixed Breed	12	Male	Neoplasia	2	143,9	8,2	1	0	1	Died

34	Miniature Poodle	7	Female	Trauma	2	110,5	12,9	2	0	2	Died
35	Great Dane	9	Male	Neoplasia	0	113,2	15,6	1	71	72	Died*
36	Mixed Breed	1	Female	Trauma	3	117	24,5	1	0	1	Died
37	Mixed Breed	11	Female	Neoplasia	1	122,3	15,1	19	18	37	Died
38	Miniature Pinscher	6	Female	Trauma	1	102	13	2	0	2	Died
39	Bull Terrier	7	Female	Autoimmune Disease	2	120	16,7	4	0	4	Died
40	Pekingese	12	Male	Neurological Disorder	2	105,9	18	1	0	1	Died
41	Mixed Breed	8	Female	Neoplasia	2	124	17,3	5	0	5	Died
42	Bullmastiff	4	Male	Urinary Tract Disease	2	104,5	15,1	3	0	3	Died*
43	Mixed Breed	5	Male	Trauma	2	110,8	16,3	1	0	1	Died

Abbreviations: y, years; qSOFA, quick sequential organ failure assessment; d, days; aPTT, activated partial thromboplastin time; PT, prothrombin time.

* = euthanised.