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Mestrado Integrado de Medicina Veterinária

A Comparison of Procalcitonin values in dogs diagnosed with sepsis versus healthy dogs

Dissertação apresentada a provas públicas para a obtenção do grau de mestre em Medicina Veterinária, orientada pelo Professor Doutor João Manuel Cardoso Martins, Doutora Joana Fonseca e Doutor José Diogo dos Santos.

Maria Inês Gameiro Borges Correia de Sousa | 21903054

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VERSÃO FINAL

Dissertação defendida por Maria Inês Gameiro Borges Correia de Sousa em provas públicas na Universidade Lusófona, Centro Universitário de Lisboa no dia 22 de Outubro de 2025, perante o júri, nomeado pelo Despacho de Nomeação n.º: 469/2025, de 25 de Setembro de 2025, com a seguinte composição:

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Este trabalho também foi orientado pela Doutora Joana Fonseca e pelo Doutor José Diogo dos Santos (co-orientadores).

Maria Inês Gameiro Borges Correia de Sousa

2025

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Resumo

A sépsis consiste em uma resposta imunológica exacerbada a uma infeção, sendo o seu diagnóstico desafiante. Como tal, o uso de biomarcadores poderá ser uma ferramenta de grande utilidade. A procalcitonina (PCT) é um biomarcador com valor diagnóstico e prognóstico em medicina humana, mas cuja evidência em cães permanece limitada.

Este estudo teve por objetivo avaliar a PCT como biomarcador de sépsis em cães, analisando também a sua capacidade de prever risco de sépsis e a sua correlação com algumas células sanguíneas. Foram incluídas neste estudo amostras sanguíneas de 51 cães, dos quais 25 saudáveis e 26 com critérios de sépsis.

As diferenças entre grupos foram avaliadas através do teste T de *Student* ou com o teste de *Mann-Whitney*. As correlações foram obtidas com os testes *Pearson's* e *Spearman's*, e a capacidade preditiva da PCT foi analisada por regressão logística binária.

Neste estudo foi proposto um intervalo de referência para os níveis séricos de PCT em cães saudáveis de 0.472 ± 0.23 ng/mL. Os resultados confirmaram o potencial da PCT como biomarcador diagnóstico de sépsis em cães, evidenciando-se uma diferença significativa entre os valores de PCT de animais saudáveis (1.28 ± 0.527) e de animais com critérios de sépsis (1.98 ± 1.19). Além disso, a PCT mostrou-se independentemente associada à presença de sépsis (OR: 2.829; 95% CI: 1.1727-6.824; P = 0.021). Observou-se ainda uma correlação entre PCT e contagem plaquetária, um achado descrito em humanos como útil na previsão de mortalidade.

Este estudo reforça a aplicabilidade clínica da PCT como biomarcador diagnóstico de sépsis em cães, ajudando a sustentar o que estudos anteriores relataram. Os resultados sugerem que a PCT pode também ter utilidade prognóstica.

Palavras-chave: cães, sépsis, biomarcadores, procalcitonina.

Abstract

Sepsis consists of an exacerbated immune response to infection, and its diagnosis is challenging. Therefore, the use of biomarkers may be a highly valuable tool. Procalcitonin (PCT) is a biomarker with diagnostic and prognostic value in human medicine, but evidence in dogs remains limited.

The aim of this study was to evaluate PCT as a biomarker of sepsis in dogs, analyzing its ability to predict the risk of sepsis, and its correlation with certain blood cells. Blood samples from 51 dogs were included in this study, of which 25 were healthy and 26 met the criteria for sepsis.

Differences between groups were assessed with Student's t-test or the Mann-Whitney test. Correlations were obtained with Pearson's and Spearman's tests, and the predictive ability of PCT was analyzed using binary logistic regression.

In this study, a reference interval for serum PCT levels in healthy dogs of 0.472 ± 0.23 ng/mL was proposed. The results confirmed the potential of PCT as a diagnostic biomarker of sepsis in dogs, showing a significant difference between PCT values in healthy animals (1.28 ± 0.527) and dogs meeting the criteria for sepsis (1.98 ± 1.19). Furthermore, PCT was independently associated with the presence of sepsis (OR: 2.829; 95% CI: 1.1727-6.824; P = 0.021). A correlation was also observed between PCT and platelet count, a finding described in humans as useful for predicting mortality.

This study reinforces the clinical applicability of PCT as a diagnostic biomarker of sepsis in dogs, supporting what previous studies have reported. The results also suggest that PCT may have prognostic utility.

Key-words: dogs, sepsis, biomarkers, procalcitonin.

List of Abbreviations, Acronyms and Symbols:

APC - Antigen-Presenting Cell

CRP – C-Reactive Protein

DAMPs - Damage-Associated Molecular Patterns

ELISA - Enzyme-Linked Immunosorbent Assays

IL - Interleukin

LPS - Lipopolysaccharides

PAMPs - Pathogen-Associated Molecular Patterns

PCT – Procalcitonin

qSOFA – Quick Sequential Organ Failure Assessment

SAA - Serum Amyloid A

SD – Standard Deviation

SIRS - Systemic Inflammatory Response Syndrome

SOFA – Sequential Organ Failure Assessment

TNF – Tumor Necrosis Factor

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I – Curricular Internship

As part of the Integrated Master's Degree in Veterinary Medicine at the Universidade Lusófona de Humanidades e Tecnologias, the author completed a six-month internship at Anicura Restelo Hospital Veterinário, from September 2024 to March 2025.

During this period, the author had the opportunity to learn from a diverse team of medical professionals in various fields of veterinary medicine. This included observing and assisting with consultations, surgeries, and diagnostic imaging procedures, as well as helping to treat and care for animals. The hospital was chosen because it is a referral center for multiple specialties. It offers exposure to a diverse range of cases and access to advanced diagnostic tools, ensuring a comprehensive learning experience.

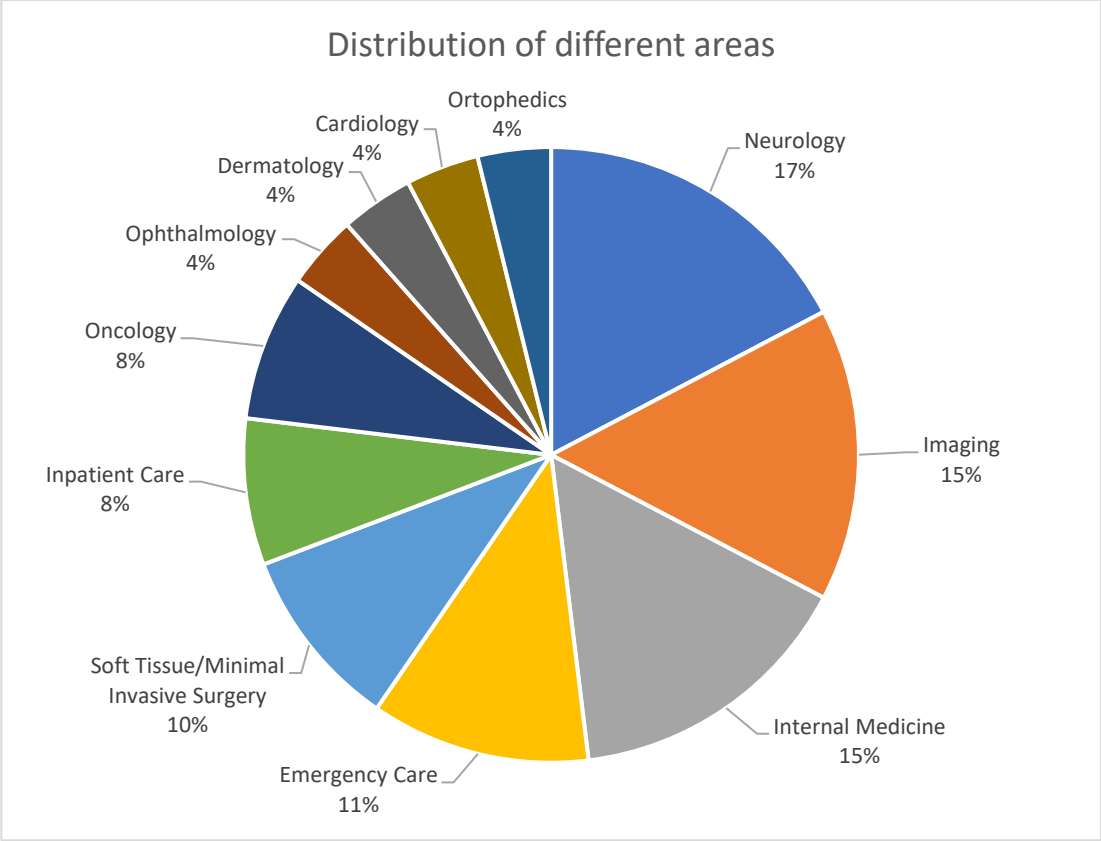
The author followed the schedule of the assigned doctors, working five shifts per week, including weekends, with each shift averaging eight hours. Shift times varied and included morning (9-17.30h), intermediate (10-18.30h), afternoon (14-19.30h), and night (20-9h). During the program, the author completed a total of 128 shifts, for a total of approximately 1040 hours.

Every two to three weeks, the author selected a different doctor from a new specialty area to accompany, allowing for focused learning through direct observation and participation in daily clinical activities. In total, the author completed eleven area-based rotations, each lasting approximately two weeks. However, there was flexibility to extend or shorten rotations based on the author's preferences. The rotations included internal medicine, neurology, oncology, inpatient care, orthopedics, and surgery (soft tissue surgery, orthopedics, and minimally invasive surgery). Other rotations included emergency and urgent care, imaging, cardiology, dermatology, and ophthalmology.

There was considerable overlap between specialties, making it a challenge to define rotations strictly. For instance, during the surgery rotation, which included soft tissue surgery and minimally invasive surgery, the author also had the chance to participate in orthopedic surgeries and orthopedic consultations. Similarly, while accompanying an ultrasound physician, the author also had the opportunity to observe several internal medicine consultations. Additionally, imaging and neurology overlapped considerably, as one of the neurologists the author accompanied was primarily responsible for magnetic resonance imaging, computed

tomography scans, and neurological examinations of hospitalized animals. However, the author also had the opportunity to join him for some neurology consultations.

The estimated distribution of the author's participation across the different service areas is shown below, in Chart 1.



Graph 1 – Distribution of the author's participation across the different areas throughout the 6 months of internship.

This rotation system allowed the author to engage in case-based learning tailored to each specialty. Shifts were distributed throughout the day to reflect the real-life dynamics of hospital work. Night shifts were exclusive to the emergency care rotation. This diversity in specialties and schedules ensured an immersive and comprehensive clinical experience.

During daily consultations, the author had the chance to observe and engage with different methodologies and approaches to each case. The author had the opportunity to learn about various diagnostic and therapeutic approaches to different situations, and was also able to observe different ways of dealing with owners and colleagues.

Under the supervision of hospital staff, the author had the opportunity to carry out various medical procedures, including blood and urine analysis, physical examinations, catheter placements, blood sample collections, medication and vaccine administrations, radiographies, neurological examinations, urinary catheterizations, fine needle aspiration cytology and fluorescein tests. On the surgery rotation, the author performed trichotomy of the surgical area and its antisepsis, endotracheal intubation, and participated in various surgeries.

During the internal medicine rotation, the author learned mainly about diagnostic approaches based on an animal's history or clinical presentation, as well as appropriate therapies for various diseases. They also gained insight into managing uncontrolled conditions and the importance of continuous monitoring, actively listening to the owner's report closely following each case. Additionally, one of the doctors that the author accompanied during this rotation also performed endoscopies, colonoscopies, rhinoscopies and bronchoalveolar lavages, procedures that the student had the opportunity to observe. The other doctor the student accompanied also performed ultrasonography, allowing them to follow cases of internal medicine alongside ultrasound evaluations. This provided valuable insight into the integration of imaging techniques with clinical assessments, enhancing the student's understanding of diagnostic processes and patient management.

During the neurology rotation, the author had the opportunity to assess and participate in the evaluation of hospitalized animals with diverse clinical presentations, interpreting their exams, to determine neurolocalization. They also observed numerous cases during consultations, learning about diagnostic and therapeutic approaches, particularly for complex, chronic and incurable conditions, such as idiopathic epilepsy. With access to computed tomography and magnetic resonance imaging at the hospital, the author was able to correlate clinical presentations with imaging findings, significantly enhancing their learning experience.

In addition to neurological cases, the author had the opportunity to observe and interpret computed tomography scans for a variety of other conditions. These included oncological staging, pre-surgical imaging for procedures such as mass excisions and renal bypasses, and the diagnosis of several conditions like ectopic ureters, hernias, and persistent right fourth aortic arch, for example. The student also followed post-surgical evaluations, including imaging assessments after hemilaminectomies, further expanding their understanding of advanced diagnostic imaging in clinical practice.

While accompanying a doctor dedicated to emergency consultations, the student learned the importance of conducting a thorough anamnesis, following a structured diagnostic approach based on the animal's history and clinical presentation, and recognizing when referral is necessary. Their understanding of the ABCDE approach in emergencies was reinforced and they also gained insight into initial therapeutic interventions for various critical situations. Additionally, the author observed effective communication strategies for interacting with owners, whether to provide reassurance during stressful moments or to sensitively prepare them for end-of-life situations, including euthanasia.

On the surgery rotation, some of the procedures the student had the opportunity to observe and/or participate were: ovariohysterectomy, orchiectomy, minimal invasive surgeries (e.g. ovariectomy, pericardiectomy), exploratory laparotomy, splenectomy, subcutaneous ureteral bypass colocation, enterectomy, cystotomy, hemilaminectomy, amputations, craniotomy, tibial plateau leveling osteotomy, tibial tuberosity transposition tool technique, video otoscopy, several biopsies (whether by endoscopy techniques or by laparotomy). The student also had the opportunity to observe various pre-surgery consultations and learned more about anesthesia and the medications that different doctors choose to use for different procedures.

During the oncology rotation, the student gained experience in staging oncological cases, implementing chemotherapy protocols, and approaching owners with empathy, particularly when treatment options become limited. They also had the opportunity to observe electrochemotherapy.

Throughout the inpatient care rotation, the student participated in case handovers and discussions. This allowed them to monitor the progress of various animals and deepen their understanding of clinical decision-making in different scenarios. The author followed cases throughout hospitalization, performing and assisting with physical examinations, blood tests, radiographies, ultrasounds, echocardiograms and other necessary diagnostic procedures. In some instances, the author also assisted in surgeries of some of the hospitalized patients. This rotation was particularly valuable for learning how to manage critically ill animals, as well as understanding the appropriate medications and supportive treatments required for each case.

In dermatology, the author worked with both common conditions, such as atopic dermatitis and external otitis, as well as more complex and rare cases like lupus erythematosus and bullous pemphigoid. Since the hospital was a referral center, it had many challenging

dermatological cases, which allowed the author to gain valuable experience with intricate treatment plans. They also had the opportunity to observe and interpret various cytological examinations.

During the cardiology rotation, the student accompanied doctors who focused solely on echocardiography, observing exams performed on both inpatients and outpatients. This experience was essential for reinforcing theoretical knowledge and gaining a deeper understanding of the technical aspects of the procedure.

Lastly, in the orthopedics rotation, the student attended numerous consultations, learning how to conduct thorough physical examinations to identify signs of osteoarticular or muscular pain, as well as any abnormalities that could explain the animal's clinical presentation. They also participated in post-surgical follow-ups and assisted in performing radiographs for both monitoring and diagnostic purposes. Additionally, the student gained valuable insight into the management of osteoarticular pain, a prevalent issue in older animals.

The author was involved in the consultations, examinations, surgical procedures, and/or overall care of a total of 624 animals, including both cats and dogs. These comprised participation in the care of 88 animals during the neurology rotation, 154 in imaging, 77 in internal medicine, 93 during emergency care, 44 in surgery, 39 in inpatient care, 36 in oncology consultations, 40 in ophthalmology, 25 in dermatology, 12 in cardiology, and 16 in orthopedics.

Throughout the internship, the author also attended multiple lectures held by some of the veterinary doctors, covering topics such as neurologic exam, veterinary point of care ultrasound, ophthalmologic emergencies, the ABCDE approach, oncologic emergencies, the importance of observation in the neurological exam and histiocytic disorders.

Throughout the internship, the student observed an average of 624 animals from various specialties.

In these six months, the author had the privilege of working alongside a highly qualified veterinary team, which played a fundamental role in applying and refining both theoretical and practical competencies. The curricular internship provided the opportunity to acquire knowledge in various medical fields, observe different work methodologies and develop essential skills by closely following experienced veterinary professionals.

Overall, the internship played a vital role in the author's development as a future veterinarian, serving as an enriching and fundamental experience for both professional and personal growth.

II – A Comparison of Procalcitonin values in dogs diagnosed with sepsis versus healthy dogs

1. Introduction

Sepsis is a life-threatening condition that occurs secondary to a host's dysregulated immune response as a consequence of an infection (Singer *et al*., 2016). Although its global significance, there is yet no validated "gold standard" diagnostic test for sepsis. Therefore, sepsis is currently identified based on a constellation of clinical signs, in a patient with suspected or confirmed infection (Singer *et al*., 2016).

Identification of infection can be challenging, and a presumptive diagnosis of sepsis based on the clinical picture may be necessary to initiate treatment (DeClue, 2017). In human medicine, biomarkers are known to be a useful tool in these situations. They are widely used to provide a more precise diagnosis, to aid in the determination of prognosis, and to enable clinicians to individualize treatment and patient care (Goggs *et al*., 2022; Goggs, 2021). In veterinary medicine there are yet no reliable biomarkers for sepsis. However, the behavior of different biomarkers, which is a subject increasingly studied, can be useful.

Procalcitonin (PCT) is an intracellular precursor of calcitonin that is released into the bloodstream in individuals with systemic bacterial infections, being used as an important sepsis biomarker in human medicine (Paudel *et al*., 2020). In veterinary medicine, there is still a very limited number of studies, and so it hasn't yet been used as a diagnostic and prognosis tool in clinical practice (Battaglia *et al*., 2020). The clinical characteristics and utilities of PCT in dogs are also not as well established as they are in humans. However, there has been an increase in PCT research in dogs in recent years (Koho *et al.*, 2024).

1.1 Sepsis

Sepsis is a life-threatening organ dysfunction with extensive physiological, immune, metabolomics, and biochemical abnormalities, that happens due to a dysregulated host response, in consequence of an infection (Jarczak *et al*., 2021; Singer *et al*., 2016). A study of human medicine showed that an estimated 48.9 million cases of sepsis were recorded worldwide in 2017, resulting in 11 million sepsis-related deaths, representing 19.7% of all

global deaths (Rudd *et al*., 2020). There are no precise estimates of the occurrence of sepsis when it comes to veterinary medicine. However, mortality rates of 20-68% have been reported (Cortellini *et al.,* 2024).

There has been a long history of attempts to define sepsis in human medicine, the latest consensus dating back to 2016 (Singer *et al*., 2016).

Previously, sepsis was considered to be a systemic inflammatory response syndrome (SIRS) associated with an infection (Bone *et al*., 1992; Levy *et al*., 2003). A list of diagnostic criteria of sepsis was made, and to attribute a diagnosis of sepsis to a patient they needed to meet some of the SIRS criteria. This concept of sepsis focuses solely on inflammatory excess (Hotchkiss *et al*., 2013). However, in recent years, sepsis has been associated not only with pro-inflammatory response but also with anti-inflammatory, as well as with several modifications in various pathways, such as cardiovascular, neuronal, autonomic, hormonal, bioenergetic, metabolic, and coagulation (Hotchkiss *et al*., 2013; Singer *et al*., 2016). Therefore, using SIRS criteria alone can raise many questions, as many of the criteria occur in most cases of inflammation, regardless of the presence of an infection (Singer *et al*., 2016).

"Today, this heterogeneous syndrome is defined as severe organ dysfunction caused by an overactive host response to an infection" (Jarczak *et al*., 2021, p. 1). Despite significant advances and the ongoing efforts in experimental and clinical research, improving the outcome in septic patients remains a challenge.

Since the publication of Sepsis-3 by Singer *et al*. (2016), the definitions of sepsis in humans and animals have diverged (Cortellini *et al*., 2024). This limits the ability to apply human medicine sepsis literature to veterinary practice.

1.2 Sepsis Pathophysiology

Unlike an uncomplicated, localized infection, sepsis is a complex disruption of the immunological balance between inflammation and anti-inflammation (Figure 1) (Jarczak *et al*., 2021).

During an infection, "the causative pathogen replicates and releases its constituents such as endotoxins, exotoxins, and DNA" (Barichello *et al*., 2022, p. 2). These components,

known as pathogen-associated molecular patterns (PAMPs), are recognized by the innate immune system through receptors on the surface of antigen-presenting cells (APCs) and monocytes (Barichello *et al*., 2022; Jarczak *et al*., 2021).

In the initial phases of infection, PAMPs induce systemic inflammation by activating local innate immune cells (DeClue, 2017). The activation of inflammatory cells results in the production and release of pro- and anti-inflammatory mediators, including tumor necrosis factor (TNF)-alpha and interleukins (IL). These mediators contribute to the activation of endothelial cells and the upregulation of adhesion molecules. Based on the inflammatory mediator signals, neutrophils, monocytes, and lymphocytes travel to the site of infection, leading to a second release wave of inflammatory mediators (DeClue, 2017).

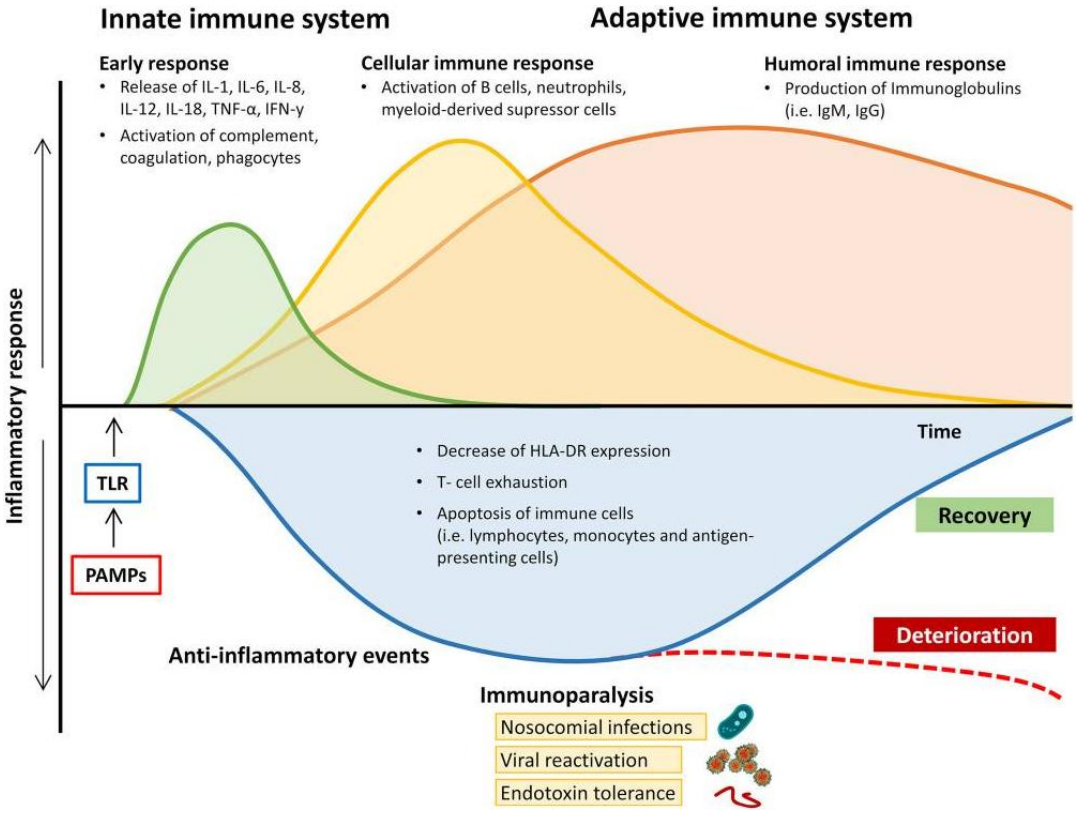


Figure 1 – Changes in pro- and anti-inflammatory response of the immune system during the course of sepsis. HLA-DR: human leukocyte antigen-D related, IgM/G: immunoglobulin M/G; IL – interleukin, IFN-γ: interferon γ, PAMPs: pathogen-associated molecular patterns, TNF-α: tumor necrosis factor alpha, TLR: toll-like receptor (Jarczak *et al*., 2021).

Neutrophils play a significant role in the initial defense against infections. However, when they are activated by PAMPs or damage-associated molecular patterns (DAMPs), immature neutrophils show reduced phagocytosis and oxidative burst capacity (Drifte *et al*., 2013). In patients who are clinically deteriorating, there is often an association with the rise of serum levels of neutrophils, which in turn is related to an increased production of neutrophil extracellular traps (NETs) (Jarczak *et al*., 2021). NETs are large extracellular structures that trap, neutralize, and kill bacteria, fungi, viruses, and parasites (Brinkmann *et al*., 2004). NETs have the potential to immobilize a wide range of pathogens, however, in sepsis, excessive NET formation and dysregulated clearance seem to be implicated in the exacerbation of sepsis, by promoting inflammation, tissue damage, and thrombosis (Czaikoski *et al*., 2016; DeClue, 2017).

The complement system is another important component of the innate immune response. It is activated in the presence of invading pathogens and/or in response to tissue damage (Markiewski *et al.,* 2008). This system has antimicrobial properties and has a role in coordinating the inflammatory process that is triggered in response to infection. Some of the complement proteins are involved in the regulation of vascular flow, blood vessel caliber, vascular permeability, and leukocyte extravasation and chemotaxis (Markiewski & Lambris, 2007). The complement proteins coordinate cellular inflammatory responses, by binding to peripheral blood leukocytes or macrophage receptors, and control cytokine production and secretion. The activation of the complement system generates a proinflammatory response that increases vascular permeability and chemotaxis of these cells, a very useful response in early innate immune responses (Ward, 2008). However, these potent proinflammatory effects, when there are uncontrolled and exacerbated infections, might induce collateral tissue damage, leading to hyperinflammation (Kyriazopoulou *et al*., 2017).

In addition to their direct role in attacking pathogens, neutrophils, monocytes, and the complement system can interact with platelets and the coagulation cascade (Maneta *et al*., 2023). This leads to a process called immunothrombosis, which involves the formation of thrombi. Immunothrombosis is a process that can be beneficial for the host, since it restricts the pathogen's spread, tissue invasion, and survival. However, during sepsis, uncontrolled immunothrombosis can trigger aberrant thrombosis in the microcirculation, resulting in disseminated intravascular coagulation (Maneta *et al*., 2023).

Microvasculature dysfunction (including vasoplegia, loss of endothelial antithrombogenicity, increased permeability, and increased vascular-blood cell interactions) is considered the direct mechanism underlying organ dysfunction in septic patients (Arina & Singer, 2021; Helms *et al.*, 2023). The microcirculatory damage is triggered by injury to the endothelial glycocalyx – a gel-like layer that covers the entire luminal surface of the vasculature, composed of glycosaminoglycans (Patterson *et al*., 2022). Being highly susceptible to damage during inflammatory responses, increased proteolytic processes activated during sepsis cause further injury (Iba *et al*., 2024b). A damaged glycocalyx results in capillary leak syndrome, which manifests as an excessive fluid shift from the intravascular to the extravascular space, resulting in hypovolemia, interstitial edema, and hypoperfusion (Saravi *et al*., 2023). The structural changes that occur due to a damaged glycocalyx lead to an exposition of adhesion molecules, which leads to blood cell adhesion and upregulate the interaction between inflammatory cells and coagulation (Iba *et al*., 2024b). Enhanced interactions between platelets and leukocytes lead to the formation of NETs, that further increase endothelial injury and thromboinflammation (Iba et al., 2024a).

Tissue factor is a protein, also called coagulation factor III, that is usually not expressed by cells exposed to the bloodstream, such as endothelial cells, in order to prevent improper coagulation cascade activation (Cimmino *et al*., 2015). However, during sepsis, endothelial cells express this protein, acquiring procoagulant and antifibrinolytic properties to prevent the dissemination of the infection (Raia & Zafrani, 2022). Tissue factor initiates the coagulation cascade, anticoagulant proteins are downregulated, and the endothelium also starts to release large amounts of plasminogen activation inhibitor, leading to an antifibrinolytic condition (Levin *et al*., 1989; Raia & Zafrani, 2022). In addition, endothelial cell damage induces the release of the von Willebrand factor, which leads to the recruitment and aggregation of platelets (Raia & Zafrani, 2022). This endothelium activation is appropriate during acute infections, however, in extreme cases such as sepsis, disseminated intravascular coagulation can occur (Iba *et al*., 2019).

APCs that have ingested a pathogen are the ones responsible for the activation of the adaptive immune response (DeClue, 2017). In response, naïve T-cells differentiate and contribute to the production of additional cytokines, either with proinflammatory or anti-inflammatory effects, depending on which type they differentiate.

There is a significant component of concomitant immunosuppression that occurs both in the early and late stages of sepsis, due to the downregulation of APCs, increased apoptosis of immune cells and T-cell exhaustion (Jarczak *et al*., 2021; Venet *et al*., 2018). The compensatory anti-inflammatory response syndrome is characterized by a relative lack of immune response or, in more severe cases, immunoparalysis (Hotchkiss *et al*., 2013). The sepsis-induced lymphopenia and the decrease of immunoglobulin levels are highly associated with increased mortality (Jarczak *et al*., 2021; Venet *et al*., 2018).

The pathophysiology of sepsis is now recognized as a continuum unbalance between a hyperinflammatory and hypoinflammatory phase (Hotchkiss *et al.*, 2013). Ultimately, an untreated hyperinflammatory phase leads to hemodynamic instability, vasodilation, vascular leakage, activation of coagulation, multiple organ failure, and so on, and, on the contrary, the hypoinflammatory phase can lead to the development of opportunistic infections (DeClue, 2017)

1.3 Diagnostic Approach to Sepsis in Human Medicine

Currently, there is no validated "gold standard" diagnostic test for sepsis (Singer *et al*., 2016). For this reason, sepsis is identified based on the clinical signs and symptoms of a patient with suspected or confirmed infection. However, there are yet no simple and explicit clinical, biological, imaging, or laboratory features that categorize a septic patient. The clinical manifestations of sepsis are also highly variable, since they depend on the initial site of the infection, the causative microorganism, the underlying health status of the patient, the interval before initiating treatment, and the pattern of acute organ dysfunction. Furthermore, the immunological phenotype depends highly on the individual affected, leading to considerable diagnostic difficulty (Jarczak *et al*., 2021).

While pathogen detection remains the most reliable method for diagnosing infections, clinical microbiological culture and identification methods are often slow, typically taking at least 2-3 days, and often failing to detect the presence of pathogens, even if the infection is present (He *et al*., 2024). The absence of early specific diagnostic markers results in many sepsis cases being diagnosed only in later stages, resulting in less optimal treatment outcomes and poorer prognosis. This emphasizes the importance of using simple protocols in intensive care

units to identify possible septic patients, using biomarkers and scoring systems to diagnose and assess the patient's status (He *et al*., 2024).

In human medicine, the use of two or more SIRS criteria to identify sepsis was considered unhelpful, since these criteria do not necessarily indicate a dysregulated response of the immune system (Singer *et al*., 2016). In addition, the study by Kaukonen *et al*. (2015) showed that 12.1% of the patients with sepsis showed less than two SIRS criteria. However, SIRS with a suspected source of infection should be taken into account, especially in early stages, where waiting for the confirmation of infection with positive cultures may be dangerous (Chakraborty & Burns, 2023). In practice, SIRS is reported to be helpful as a triage tool to identify potentially septic patients. However, once it's identified, other scores should be used to assess illness severity and the need for intensive care intervention (Gunn *et al.,* 2016).

The article by Chakraborty & Burns (2023) explains that nearly all septic patients have SIRS, but not all SIRS patients are septic. Therefore, other scores are needed for a more accurate diagnosis of sepsis.

Often, early sepsis manifests as reduced capillary refill time, mottled skin, tachypnea, and altered mental status (Arora *et al*., 2023). After the onset of circulatory failure, the thrombus formation results in tissue hypoperfusion, which is aggravated by hypotension (Opal & van der Poll, 2014). This will eventually be followed by shock and multiple organ dysfunction.

Organ dysfunction can manifest to varying degrees (Singer *et al*., 2016). In mild cases, it may not manifest clinically, therefore, its presence should be considered in any infected patient. The degree of organ dysfunction is determined by the Sequential Organ Failure Assessment (SOFA) score, a tool used to characterize septic patients, not for patient management (Table 1).

Table 1 – the original SOFA score; ** adrenergic agents administered for at least 1 hour (doses given are in mcg/kg/day) (adapted from Vincent *et al*., 1996).

Organ System, Measurement	SOFA score				
	0	**1**	**2**	**3**	**4**
Respiration - PaO2/FiO2: mmHg	Normal	<400	<300	<200 (with respiratory support)	<100 (with respiratory support)
Coagulation – Platelets: $x10^3/mm^3$	Normal	<150	<100	<50	<20
Liver – Bilirubin: mg/dL	Normal	1.2-1.9	2.0-5.9	6.0-11.9	> 12.0
Cardiovascular – Hypotension	Normal	MAP < 70 mmHg	Dopamine ≤ 5 or dobutamine (any dose) **	Dopamine > 5, epinephrine ≤ 0.1 or norepinephrine ≤ 0.1	Dopamine > 15, epinephrine > 0.1 or norepinephrine > 0.1
Central Nervous System – Glasgow Coma Score	Normal	13-14	10-12	6-9	< 6
Renal – Creatinine: mg/dL or Urine Output	Normal	1.2-1.9	2.0-3.4	3.5-4.9 or <500 mL/day	> 5.0 or <200 mL/day

The baseline SOFA score can be assumed as zero when patients don't have known pre-existing organ dysfunction, and organ dysfunction can be identified when there is an acute change in the total SOFA score (≥2 points), in the presence of a presumed infection (Singer *et al*., 2016).

A faster and simpler way to identify organ dysfunction is using the quick SOFA score (qSOFA), which is based solely on the alteration of the mental status, systolic blood pressure (≤ 100 mmHg), and respiratory rate (≥ 22 breaths per minute) (Singer *et al*., 2016). A positive qSOFA criterion, implying the presence of 2 or more qSOFA points, is also useful to identify possible infections in patients who weren't previously recognized with one (Singer *et al*., 2016; Song *et al*., 2020a).

Several recent studies have questioned whether the qSOFA score is more accurate for predicting outcomes than the SIRS criteria. Nevertheless, the SIRS criteria are still commonly used in emergency rooms as part of the intensive care unit protocol (Marik & Taeb, 2017).

It is important to understand that the qSOFA, SOFA, and SIRS criteria are not intended as stand-alone definitions of sepsis. Failure to meet any of these criteria should not delay an infection's investigation or treatment, if medical professionals deem it (Marik & Taeb, 2017; Singer *et al*., 2016).

1.3.1 Sepsis Biomarkers

A biomarker, short for "biological marker", is a measurable indicator that reflects biological status in normal and/or pathogenic processes that offer utility for diagnosis, prognosis, early disease recognition, risk stratification, and deciding the appropriate treatment (Barichello *et al*., 2022). A biomarker should be accurate and reproducible, and, in the ideal scenario, it should offer both high specificity and sensitivity for diagnosing a condition.

"Over 250 sepsis biomarkers have been identified in recent years" (He *et al.,* 2024, p. 5). Sepsis biomarkers have gained significant attention due to their potential in improving early diagnosis and, consequently, prognosis of sepsis (He *et al.,* 2024). During sepsis, host biomarkers can play a critical role in the diagnosis, early identification of organ dysfunction, risk stratification, prognosis indication, and patient management (Barichello *et al*., 2022).

Researchers have studied each inflammatory response during SIRS and sepsis, studying the different metabolites associated with the inflammatory cascade and cellular components that might be used as biomarkers (Barichello *et al*., 2022). These may help to identify the presence of infection, endothelial damage, intestinal permeability, organ failure, and blood-brain barrier breakdown. These biomarkers include acute-phase proteins (e.g. C-reactive protein (CRP), albumin), cytokines, chemokines, DAMPs, endothelial cell markers, glycocalyx fragments, leukocyte surface markers, and procalcitonin (PCT) (Barichello *et al*., 2022; Uchimido *et al*., 2019).

One of the most widely used biomarkers is the CRP, whose rise is primarily induced by IL's during the acute phase of an inflammatory process (Barichello *et al*., 2022). Pentraxin-3 is another biomarker currently used that is secreted by numerous cells (e.g. macrophages, dendritic cells) under inflammatory stimulus (Erreni *et al*., 2017). Amongst the

proinflammatory cytokines, some of them are increased in critically ill patients, compared to non-critical, making them useful biomarkers for identifying a hyperinflammatory state (Matsumoto *et al*., 2018). Some DAMPs, like calprotectin, a protein found in the cytosol of neutrophils and macrophages that is released in cases of cell stress or damage, can be measured to aid in the assessment of prognosis (Larsson *et al*., 2020). Syndecan-1, a structural component of the endothelium glycocalyx, was shown to be increased in septic patients as well, in comparison to healthy controls (Barichello *et al*., 2022; Uchimido *et al*., 2019). PCT is a biomarker produced directly by stimulating bacterial components or induced by inflammatory mediators, such as IL-6 and TNF-α, and is widely used to identify possible bacteremia (Leli *et al*., 2016). Elevated lactate levels reflect either increased production by hypoperfused tissues or inadequate clearance due to liver dysfunction, making it a valuable marker aiding in predicting the clinical progression (Chakraborty & Burns, 2023).

A study by Song *et al*. (2020b) showed that the combined use of Pentraxin-3, IL-6, PCT, and lactate amongst patients diagnosed with sepsis or septic shock exhibited not only excellent performance but also outperformed the SOFA score in predicting mortality. As such, these and other biomarkers should be seen as complementary tools to support sepsis diagnosis. Nevertheless, despite their demonstrated value, their use must still be guided by medical reasoning.

1.4 Sepsis in Veterinary Medicine

"The current definitions of SIRS and sepsis used in veterinary medicine are derived from those originally published in human medicine" (Sharp, 2019, p. 1030).

SIRS is described as the clinical manifestations of a complex physiologic response to a nonspecific insult, of either infectious or noninfectious origin (DeClue, 2017). SIRS diagnosis is based on the fulfillment of at least 2 of the following clinical criteria: tachycardia, tachypnea, hypo- or hyperthermia and leukocytosis, leukopenia or >5% bands (Table 2). SIRS's criteria aim is to indicate patients that are systemically unwell and in need of prompt attention; thus, the exact cut-off points for these parameters are less relevant than the overall clinical context (Sharp, 2019).

Table 2 – SIRS criteria (adapted from DeClue, 2017)

SIRS criteria	
Tachycardia	> 140 beats per minute
Tachypnea	> 40 breaths per minute
Body Temperature	< 37.2ºC or > 39.2ºC
Leukocyte count	> 19500/µL, < 5000/µL or > 5% bands

Sepsis in veterinary medicine is characterized as a systemic inflammatory response to infection or life-threatening organ dysfunction caused by a dysregulated host response to infection (Cortellini *et al*., 2024; Singer *et al.*, 2016).

In companion animals, gram-negative bacterial infections are the leading cause of sepsis, with *Escherichia coli* being the most frequently isolated pathogen (DeClue, 2017). In dogs, sepsis most commonly originates from the abdomen and respiratory tract, although the reproductive tract also plays an important role in its development, especially in cases of pyometra (DeClue, 2017; Laforcade, *et al*., 2008).

1.5 Current Diagnostic Approach to Sepsis in Veterinary Medicine

Although equivalent evidence is lacking, delayed diagnosis and treatment likely contribute to worsened outcomes in sepsis patients, as in human medicine (Cortellini *et al*., 2024). There is still no gold standard diagnostic test for sepsis, nor sufficient research that sustains the use of specific exams to identify septic dogs. For bacterial sepsis, culture and susceptibility testing are ideal for confirming bacterial infection; however, it's important to recognize that not all organisms can grow successfully in the laboratory setting (Stewart, 2012).

In any critical patient, a complete patient evaluation should be performed, including history, physical examination, blood pressure, complete blood count, serum biochemical profile, and appropriate diagnostic imaging (DeClue, 2017). To diagnose sepsis, there should also be evidence of infection.

In some cases, identifying an infection can be difficult, so a presumptive diagnosis should be made based on the clinical picture, since delaying the treatment can severely worsen the outcome (Oduncu *et al*., 2021). The complexity and heterogeneity of sepsis make rapid diagnosis and treatment difficult, which makes the use of scoring systems, similar to those used in human medicine, highly valuable.

SIRS criteria and qSOFA score are two adjunctive scoring tools that are widely used for their simplicity and quickness (Çolakoğlu & Karaca, 2025). SIRS criteria are no longer commonly used in most cases in human medicine, since the new sepsis definition includes the application of the SOFA/qSOFA score instead (Singer *et al*., 2016). However, since validated corresponding sepsis criteria are not available in veterinary medicine, the SIRS criteria remain to be used for sepsis diagnosis.

In human medicine, many researchers have studied the performance of qSOFA and SIRS scores. Results differ from study to study, where qSOFA sometimes appears to have higher sensitivity but lower efficiency. In other cases, the opposite occurs, where SIRS appears to have the highest sensitivity (Çolakoğlu & Karaca, 2025). A study in veterinary medicine by Çolakoğlu & Karaca (2025) showed that the qSOFA score had a good performance when it came to the diagnosis of pyometra, yet appeared to have lower efficiency in comparison to the SIRS score. Either way, it is important to recognize that, in sepsis, high sensitivity is more important than specificity, since the risk of wrongly diagnosing sepsis is much less catastrophic than missing a real case (Goulden *et al*., 2017).

The study by Çolakoğlu & Karaca (2025) concluded that the use of both scores appeared to provide a more accurate result in the diagnosis of sepsis. They can’t be the only tests used to diagnose sepsis, but they can help guide the diagnosis and treatment.

SIRS and sepsis in dogs have multiple clinical manifestations, and these can be classified into hyper- and hypodynamic phases (Silverstein, 2014). On one hand, the hyperdynamic phase is characterized by bounding pulses, congestive mucous membranes, fever, tachycardia, and tachypnea. The hypodynamic phase, on the other hand, is considered a more advanced clinical scenario, being characterized by hypotension, hypothermia, pale mucous membranes, and weak pulses. Many animals with SIRS or sepsis, present with a history of diarrhea, lethargy, loss of appetite, change in mentation and/or vomiting (Silverstein, 2014).

Analytically, leukocytosis or leukopenia, neutrophilia with a left shift or neutropenia, anemia, thrombocytopenia, hyper- or hypoglycemia, hypoalbuminemia, azotemia, hyperbilirubinemia, increased alanine aminotransferase, and increased alkaline phosphatase concentrations are all alterations recognized during sepsis (DeClue, 2017; de Laforcade, 2010).

Sepsis diagnosis requires meeting ≥ 2 SIRS criteria along with confirmed or strongly suspected infection (Sharp, 2019). The qSOFA score is used mainly to help identify organ damage, in which a score of at least 2 is associated with increased mortality (Çolakoğlu & Karaca, 2025). The qSOFA score parameters used in veterinary medicine are based on the article by Singer *et al.* (2016) (Table 3).

Table 3 – qSOFA score parameters (adapted from Singer *et al*., 2016)

qSOFA score	
Respiratory rate	1 point if ≥ 22 breaths per minute, 0 point if < 22 breaths per minute
Mental status change	1 point if there's any change, 0 if there isn't
Systolic blood pressure	1 point ≤ 100 mm/Hg, 0 points if ≥ 100 mm/Hg

Organ dysfunction may develop due to sepsis and it can be identified using established criteria (SOFA score) (Sharp, 2019). Multiple organ dysfunction syndrome is diagnosed when an animal exhibits ≥ 2 forms of organ dysfunction in the presence of relevant risk factors (Table 4) (DeClue, 2017).

Table 4 – Definition of Organ Dysfunction in Dogs (adapted from Kenney *et al*., 2010)

Organ/System	Criteria
Renal	An increase in creatinine concentration ≥ 0.5 mg/dL from presurgical values without evidence of pre- or postrenal azotemia
Cardiovascular	Hypotension sufficiently severe to require vasopressor therapy
Respiratory	Need for supplemental oxygen administration or mechanical ventilation; determined based on clinical assessment, blood gas analysis (alveolar-arterial gradient > 10 mmHg) and/or results of pulse oximetry (SpO2 < 95%)
Hepatic	Plasma or serum total bilirubin > 0.5 mg/dL
Coagulation	PT or PTT > 25% above the upper reference limit and/or platelet count ≤ 100.000/mcL

Septic effusion is most often linked to gastrointestinal perforations or ruptures, and to identify whether an effusion is septic or not, cytologic examination is highly valuable (Bonczynski *et al*., 2003). Measuring peritoneal fluid glucose levels can aid in the diagnosis as well, since levels of at least 20 mg/dL lower than those in the blood may indicate bacterial presence. Additionally, fluid lactate is typically higher in septic effusions compared to non-septic ones. However, neither of these measurements should be interpreted in isolation.

As previously mentioned, sometimes, identification of infection can be difficult or delayed, and a presumptive diagnosis of sepsis based on the clinical picture can be necessary (DeClue, 2017). Since rapid diagnosis is critical, as timely intervention improves clinical outcomes, it may be useful to use sepsis biomarkers, since some provide high diagnostic accuracy, while offering relatively quick and accessible results (Cortellini *et al*., 2024).

Nevertheless, it is important to interpret both scoring systems and biomarkers alongside the patient's clinical history, presentation, and medical reasoning.

1.5.1 Sepsis Biomarkers in Veterinary Medicine

Biomarkers are a useful tool in human medicine for diagnosing sepsis and determining prognosis (Goggs *et al*., 2022; Goggs, 2021) They also enable clinicians to individualize treatment and patient care. In veterinary medicine, there are yet no reliable biomarkers for

sepsis, however, the behavior of different biomarkers, which is a subject increasingly studied, can be useful in these situations.

Some examples of biomarkers that can be used in cases of sepsis are acute phase proteins (e.g. serum amyloid A (SAA), CRP), cytokines, chemokines, vascular endothelial growth factor, plasminogen activator inhibitor 1, and von Willebrand factor (Gaudette *et al*., 2023; Goggs, 2021).

Acute phase proteins, such as SAA and CRP, are increased in dogs with sepsis but do not hold prognostic value (Goggs, 2021). As being part of the innate immune response, CRP is rapidly synthesized in the event of an infection, and its value correlates with the degree of inflammation. SAA is also a protein that responds quickly when facing an infection, making it a valuable biomarker. These proteins, however, do not differentiate between infectious and non-infectious inflammation (Gebhart *et al*., 2009; Goggs, 2021; Jitpean *et al*., 2014).

Cytokines are becoming increasingly relevant as immunophenotyping is recognized, whereby an individual may exhibit a hyperactive immune response compared to immunosuppressed individuals (Goggs, 2021). This variation in immune response may influence treatment; therefore, profiling cytokines and chemokines may provide insight into a patient's immune state, aiding in therapeutic decisions.

High plasma vascular endothelial growth factor levels are associated with worse clinical outcomes in septic patients, and can be released secondary to inflammatory cytokines and bacterial elements (Gaudette *et al*., 2023). Vascular endothelial growth factor plasma levels don't appear to be related to mortality; however, studies suggest that this biomarker may play a role in the morbidity of sepsis.

Due to endothelium damage, there is production of procoagulant mediators, such as tissue factor and von Willebrand factor, and there is inhibition of fibrinolysis, via production of plasminogen activator inhibitor 1 (Opal & van der Poll, 2014). These are especially important to identify/predict disseminated intravascular coagulation.

PCT is a sepsis biomarker used in human medicine that has been reported to be more specific to bacterial infections (Goggs, 2021). Increased plasma values are associated with the worst prognosis and are used to manage antibiotic therapy. Despite its limited use in veterinary medicine, due to a lack of supporting evidence, its value and usefulness in human medicine suggest it could hold potential for veterinary applications as well.

1.6 Procalcitonin

PCT is an intracellular precursor of calcitonin, first discovered in 1975 (Paudel *et al*., 2020). This protein isn't normally secreted into the bloodstream of healthy individuals and its increase was first associated with medullary thyroid and small cell lung carcinoma in humans (Allison *et al*., 1981; Cate *et al*., 1986). It was only associated with bacterial infections in 1993. Since then, it has been used as an important biomarker for sepsis in human medicine (Assicot *et al*., 1993). In veterinary medicine, the clinical application of PCT remains under investigation, with research in dogs emerging only recently.

1.6.1 Calcitonin and Procalcitonin Physiology

Calcitonin is a hormone that is mainly synthesized by the parafollicular cells, or C cells, of the thyroid gland, and its main function is to reduce serum calcium concentrations (The Editors of Encyclopaedia Britannica [TEEB], 2022). It reduces it by inhibiting osteoclastic activity in bone tissue, preventing bone resorption, and promoting renal excretion of calcium. It also promotes the excretion of phosphorus by the kidney.

Calcitonin secretion is controlled by calcium concentrations, where high serum calcium concentrations lead to an increased calcitonin secretion (Petroff & Greco, 2020). Gastrointestinal hormones (gastrin, cholecystokinin, secretin and glucagon) also stimulate the secretion of this hormone, with gastrin having the greatest effect.

Under normal homeostasis, pre-procalcitonin is produced by the thyroid C-cells, being afterwards transformed by an endopeptidase into PCT (Paudel *et al*., 2020). It is then broken down to form N-terminal PCT, C-terminal katacalcin, and active calcitonin, by the enzyme prohormone convertase (Figure 2) (Cleland & Eranki, 2023; Duan *et al*., 2021; Paudel *et al*., 2020). Typically, in healthy individuals, PCT blood levels are less than 0.05 ng/mL, however, in cases of circulating endotoxins (lipopolysaccharides (LPS)), an extra-thyroid synthesis of PCT occurs in several organs, such as liver, lung, pancreas, kidney and intestine, as well as in leukocytes (Cleland & Eranki, 2023; Lippi & Sanchis-Gomar, 2017).

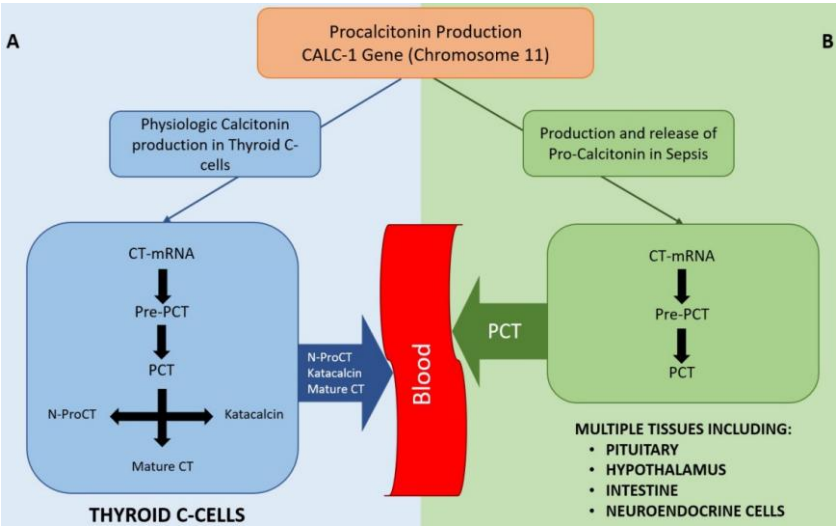


Figure 2 – Diagram of the physiology of calcitonin (A), and the pathophysiology of procalcitonin (B) during sepsis states. CT-mRNA: Calcitonin messenger RNA, Pre-PCT: Pre-procalcitonin, PCT: Procalcitonin, N-ProCT: N terminal Procalcitonin, CT: Calcitonin (Paudel *et al*., 2020).

Endotoxins are considered one of the most powerful bacterial inducers of cytokines (Cavaillon, 2018). These cytokines, such as IL-6, tumor necrosis factor and IL-1b, lead to PCT release by increasing calcitonin-1 gene transcription and, since the enzyme responsible for the degradation of PCT into calcitonin is not present in parenchymal tissues, PCT is released into the bloodstream (Linscheid *et al*., 2004; Matur *et al*., 2017). In these situations, occurring mainly in cases of systemic infections and sepsis, the PCT serum concentration can be increased up to 100-1000 times (Lippi & Sanchis-Gomar, 2017).

It is important to note that PCT serum levels are significantly lower in fungal infections compared to bacterial infections, and this biomarker is even suppressed during viral infections, due to cytokines released that down-regulate PCT levels - such as interferon-gamma (He *et al*., 2021; Matur *et al*., 2021; Meisner, 2014). It is important to keep in mind cases of mixed infections where PCT levels may be elevated due to the bacterial component, however, the magnitude of PCT production may be lower (Becze *et al*., 2016). Also, PCT levels are not particularly elevated in local bacterial infections, some not even inducing PCT synthesis (Matur *et al*., 2017).

Additionally, PCT levels in human medicine seem to correlate not only with bacterial load, increasing as the load rises, but also with the causative agent (Matur *et al*., 2017). Gram-negative bacteria, for example, lead to more PCT release than gram-positive bacteria, since each

kind of bacteria leads to the production of different inflammatory cytokines. Furthermore, the study by Delèvaux *et al*. (2003) reported that human serum PCT levels were higher in infections due to *Escherichia coli*, *Pneumocystis carinii*, *Leptospira* and *Pseudomonas aeruginosa*, moderate in infections due to *Chlamydia*, *Streptococcus pneumoniae* and *Staphylococcus aureus* and were lower in infections due to *Coxiella burnetti*, *Citrobacter freundeii* and *Salmonella*.

1.6.2 Procalcitonin as a Biomarker of Sepsis

In human medicine, PCT was found to be detectable in the serum within a few hours (2-4h) after the onset of bacterial infection, reaching its peak within 24h (Becze *et al*., 2015). The half-life of PCT is approximately 25-35h, and although its elimination pathway is still not defined, renal elimination doesn't seem to be its major mechanism, affecting only mildly its clearance when there is kidney disease (Grace & Turner, 2014). However, recent studies have found a more significant correlation between renal function and PCT values, which might suggest that severely impaired kidney function does elevate PCT levels (Wu *et al*., 2019).

Some baseline standard PCT values aid in the diagnosis of sepsis in human medicine: it is defined as sepsis when the value is between 2-10 ng/mL, severe sepsis when it's above 10 ng/mL and when it's below 0.5 ng/mL there is indication that there is not sepsis or systemic infection (Chivate *et al*., 2016).

The study of the evolution of serum PCT in a patient is also of great value, since the continuously decreasing PCT levels indicate a better prognosis, even if the peak value was previously very high (Meisner, 2014). When treatment is effective, PCT levels decrease by approximately 50% per day, and on the contrary, if the treatment is not appropriate, PCT levels may remain elevated or potentially increase. This makes PCT useful not only in aiding the diagnosis of sepsis but also in assessing the patient's prognosis and monitoring and guiding the antimicrobial treatment. In the absence of ongoing sepsis, PCT values usually normalize within 2-3 days (Schneider & Lam, 2007).

In clinical practice, PCT is mainly used in human medicine for the diagnosis and management of sepsis (Chivate *et al*., 2016). However, it's used in many other clinical conditions such as differentiating between infectious and sterile necrosis in acute pancreatitis, determining the etiology of idiopathic infectious diseases with high fever (e.g. meningitis),

identifying whether an agent is bacterial or viral in autoimmune diseases, monitoring immunosuppressed patients and monitoring patients after a transplantation.

1.7 Current Procalcitonin Research in Veterinary Medicine

In veterinary medicine, the number of studies on PCT is very limited, so it hasn't yet been used as a diagnostic or prognostic tool in clinical practice (Battaglia *et al*., 2020). The clinical characteristics and utility of PCT in dogs are not as well-established as they are in humans (Koho *et al*., 2024). However, an increasing amount of research on this topic has become available in recent years.

PCT is expressed by the same gene (calcitonin-1) in dogs as in humans, and Giunti *et al*. (2010) demonstrated that, in healthy dogs, calcitonin-1 is expressed only in the thyroid gland as well. In dogs with signs of SIRS, this gene was found to be expressed in the spleen, lung, and liver. Some incompatibilities about the gene expression were reported, though, where one dog with a non-infectious anti-inflammatory response had calcitonin-1 expression not only in the thyroid gland but also in the spleen and lung tissues (Giunti *et al*., 2010).

Serum PCT levels have only been measured a few times in dogs, so there are yet no established reference ranges. Another limitation is that, until recently, there was no validated method to measure PCT concentrations in plasma (Battaglia *et al*., 2020).

Recent studies measured the PCT values of dogs using a species-specific recombinant Enzyme-Linked Immunosorbent Assay (ELISA) kit, and although some inconsistencies were found, it is important to establish a reference range. The following reference range values were observed in previous studies: 0.05 ng/mL ± 0.0375 ng/mL (Koho *et al*., 2024); 1.195 ng/mL, range of 0.508-2.564 ng/mL (Rompf *et al*., 2021); median 0, range < 0.11 ng/mL – to 0.6160 ng/mL (Battaglia *et al*., 2020); 0.058-0.911 ng/mL (Easley *et al*. 2020); median 0.498 ng/mL, range 0.362-0.637 ng/mL (Goggs *et al*., 2018); 0.215-0.887 ng/mL, median 0.416 ng/mL (Troia *et al*., 2018).

The study by Battaglia *et al*. (2020) used the newly commercially available ELISA kit targeted for dogs (cPCT, TSZ ELISA, Framingham, MA, USA) to detect plasma PCT levels and tried to validate the kits by using species-specific recombinant PCT (rcPCT, Biovendor

Asheville, North Carolina, NC, USA). It was reported that plasma PCT concentrations measured by the rcPCT ELISA kit were statistically higher in sick (median of 7.252 ng/mL, range 0.793–9.447 ng/mL) than in healthy dogs (median 0, range < limit of detection (LOD) – it was 0.11 ng/mL – to 0.6160 ng/mL) ($p < 0.03$). However, the cPCT ELISA showed no significant statistical differences between healthy (median 2.25 ng/mL, range < LOD-117.8 ng/mL) and sick dogs (median 0, range < LOD – 78.3 ng/mL) ($p > 0.05$). This study showed that the cPCT ELISA kit was not able to detect and measure canine recombinant PCT, showing that this test could not be validated; however, it confirmed that the rcPCT is an assay that can be used to measure plasma PCT concentrations in dogs. Simultaneously, this study helped to demonstrate that dogs with sepsis have increased concentrations of PCT compared with healthy dogs.

The study by Ahn *et al.* (2021) demonstrated that the PCT levels significantly decreased after an ovariohysterectomy in dogs diagnosed with pyometra, although the PCT values did not differ much from those of healthy dogs. However, this may be explained by the fact that the analyzed cases did not develop severe sepsis. The behaviour that the biomarker showed reinforced the hypothesis that PCT can be a relevant biomarker for organ dysfunction and disease severity in dogs.

Easley *et al*. (2020) performed a study using the rcPCT ELISA kit in healthy dogs with induced endotoxemia, administering LPS originating from *Escherichia coli*. The researchers compared PCT plasma values before and after LPS injection and reported that LPS administration led to a rapid increase in PCT levels within 2-4h after administration. The PCT value remained above the baseline (the reference value used was 0,058-0,911 ng/mL) for at least 12 hours, returning to it by 48 hours after the administration of the endotoxin (Figure 3). This study demonstrated that PCT expression in dogs is quite similar to the behaviour in humans, bringing evidence that PCT may be a useful biomarker in veterinary medicine.

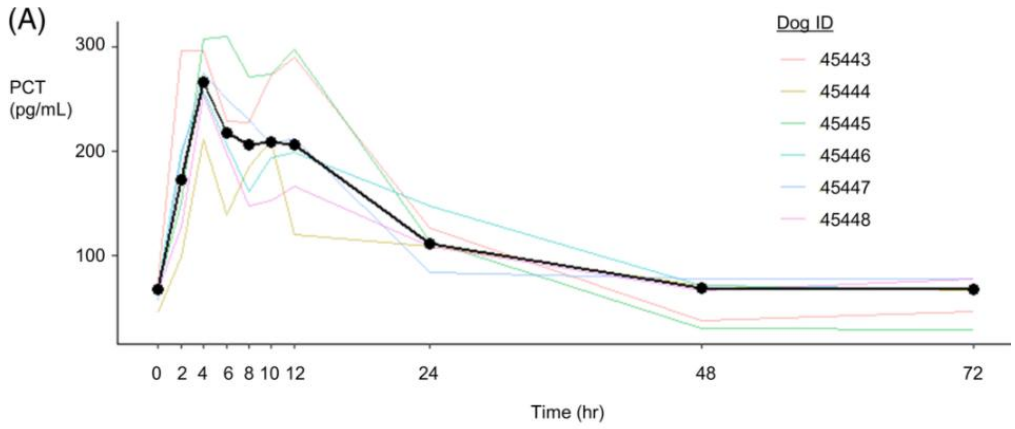


Figure 3 – Concentrations of serum procalcitonin (pg/mL) in healthy dogs before and after injection of lipopolysaccharides. Colors represent serum PCT concentration for each dog and the black line shows the median procalcitonin concentration (Easley *et al*., 2020).

The research by Goggs *et al*. (2018) compared the values of plasma PCT in healthy dogs with dogs with sepsis and gastric dilation volvulus syndrome using the rcPCT. The diagnosis of bacterial sepsis was based on the 2001 consensus definition of sepsis, by Levy e*t al.* (2003), and used established canine SIRS criteria combined with the presence of highly suspected or confirmed bacterial infection. The LOD of this study was of 0.034 ng/mL and it was reported, once again, that dogs with sepsis had higher PCT values (median 0.787 ng/mL, range 0.0391-1.647 ng/mL) than healthy dogs (median 0.498 ng/mL, range 0.362-0.637 ng/mL) ($p = 0.019$). The overlap of values may be due to the variation in illness severity of the dogs with sepsis since, as previously discussed, PCT values correlate with the bacterial load (Matur *et al*., 2017).

In the study by Troia *et al.* (2018), also using the rcPCT from Biovendor®, it was reported a range of PCT concentrations of 0.154- 4.702 ng/mL (median 1.030 ng/mL) in dogs with sepsis and 0.215-0.887 ng/mL (median 0.416 ng/mL) in healthy dogs ($p < 0.001$). As seen in the study by Goggs *et al*. (2018), there is an overlap of values, which can again be explained by the variation in illness severity.

Despite the research presented above, some studies bring doubt on the actual relevance of this biomarker. The study by Koho *et al*. (2024) measured the PCT serum concentrations in healthy dogs and in dogs with bacterial lower respiratory tract infections, reporting that the infected animals did not have an elevated PCT concentration. This could be because most of the animals that participated in this study had a local infection and, as previously said, PCT

levels are not particularly elevated in local bacterial infections (Matur *et al*., 2017). Also, in the study by Goggs *et al*. (2018), dogs with gastric dilation volvulus syndrome appeared to not have values significantly different compared to healthy dogs ($p = 0.072$) nor dogs with sepsis ($p = 1.000$) (median 0.603 ng/mL, range 0.433-1.372 ng/mL) but, this could be explained by the fact that dogs with gastric dilation are commonly affected by SIRS but do not necessarily develop sepsis, although some might do. Although this study chose dogs with gastric dilation volvulus syndrome without sepsis suspicion, it could have been present sub-clinically.

1.8 Considerations for the Clinical Use of Procalcitonin

As previously mentioned, it should always be considered that the PCT levels vary with the illness severity, bacterial load, site of infection and with the pathologic agent responsible for the infection.

It is also important to recognize that, when there is no perceived risk of bacterial diseases, PCT should not be measured, since it can be falsely elevated, and that could lead to unnecessary antibiotic use (Katz *et al*., 2019). There have been various causes described to originate falsely elevated values of PCT: newborns with less than 48-72h of age, massive stress (e.g. severe trauma, major surgery, cardiac shock or major burns), malaria, some fungal infections, prolonged and severe cardiogenic shock or organ perfusion abnormalities, treatment with medications that stimulate cytokine release, systemic vasculitis, acute graft vs host disease, acute sterile pancreatitis, heatstroke, rhabdomyolysis, end-stage renal disease, acute liver failure and paraneoplastic syndromes (e.g. medullary thyroid carcinoma, small cell lung cancer, bronchial carcinoma or neuroendocrine tumors of the gastrointestinal system) (Becze *et al*., 2016; Dornbusch, *et al*., 2008; Katz *et al*., 2019;Sugihara *et al*., 2017). However, in these conditions, usually, the PCT levels show a rapid decline compared to cases of bacterial infection (Meisner, 2002).

There has been no evidence that PCT levels are affected by glucocorticoids, non-steroidal anti-inflammatory drugs (NSAIDs), neutropenia, immunosuppression, viral diseases and kidney disorders (Chivate *et al*., 2016). Age, body weight and storage time of the sample were reported not to affect PCT concentrations as well (Koho *et al*., 2024; Rompf *et al*., 2021).

The purpose of this study was to improve the understating of the role of PCT as a sepsis biomarker, and its applicability in animals suspected or diagnosed with sepsis. The objectives of this study were: 1) to analyze differences in leukocytes, neutrophils, lymphocytes, platelets, and PCT between healthy and septic animals; 2) to contribute to the establishment of a reference range for serum PCT concentrations in healthy dogs; 3) to evaluate the predictive value of PCT for sepsis in dogs; and 4) to evaluate the correlation between PCT and leukocytes, neutrophils, lymphocytes, and platelets.

2. Materials and Methods

This study was submitted and validated by the Ethics and Animal Welfare Committee of the Faculty of Veterinary Medicine at Lusófona University under process number 29-2024.

2.1 Study Population and Sampling Institutions

An a priori sample size calculation was performed using the G*Power 3.1.9.7 statistical program. With an alpha value of 0.05, an odds ratio of 2.33, and a power of 80%, a minimum of 52 animals were required to evaluate PCT.

Blood samples were collected from a total of 51 dogs in AniCura Restelo Veterinary Hospital, Universidade Lusófona Teaching Hospital, and AniCura Vasco da Gama Veterinary Hospital. Samples were collected from October 2024 to April 2025, based on the casuistry of each institution. The PCT analysis was conducted by the Clinical Analysis Laboratory team, of the Faculty of Veterinary Medicine - Lusófona University.

Blood samples were collected from 26 dogs that were diagnosed with or were suspected of having sepsis (Septic group). These animals were selected based on the patient's history, which should indicate the presence of systemic bacterial infection, and the fulfillment of at least 2 SIRS criteria. The other group (Healthy group) was made up of 25 healthy dogs that presented to the hospital for elective surgeries (ovariohysterectomy, ovariectomy, and orchiectomy), all with no alterations in the physical and analytical exams.

2.1.1 Inclusion and Exclusion Criteria

No criteria were applied based on the dog's age, breed, sex, or weight in either group, except that newborns and dogs weighing less than two kilograms were excluded.

In the Septic group, inclusion criteria comprehended animals that had an history that correlated with systemic bacterial infection, such as animals that suffered some trauma with large exposed wounds (e.g. bite wounds, run overs, exposed fractures), animals diagnosed with sepsis, by a positive microbiological blood culture, or animals with clinical manifestations that could correlate with sepsis, whether compatible with the hypodynamic or hyperdynamic phase. As previously discussed, the hyperdynamic phase is characterized by strong pulses, congested mucous membranes, fever, tachycardia, and tachypnea (Silverstein, 2014). In contrast, the hypodynamic phase is characterized by hypotension, hypothermia, pale mucous membranes,

and weak pulses. However, it is essential to note that these phases can overlap significantly (Tsirigotis *et al*., 2016).

The exclusion criteria of Septic group consisted of factors that could affect the serum PCT concentrations: (1) dogs with only focal infections or suspected viral and/or fungal infections; (2) severe dehydration (> 10%) (Table 5); (3) dogs that started antibiotic therapy for more than 24h prior sampling, since the levels of PCT may be decreased; (4) situations that interfere with PCT levels, such as major surgery, severe trauma, severe burns, and prolonged cardiogenic shock (Becze *et al*., 2016; Dornbusch*, et al*., 2008; Sugihara *et al*., 2017).

Table 5 - Subjective estimation of percentage dehydration based on physical examination parameters (adapted from Lyons & Waddell, 2017).

Estimated Dehydration	Physical Examination Findings
< 5%	Not detectable
5% - 6%	Sticky mucous membranes
6% - 8%	Decreased skin elasticity and dry mucous membranes
8% - 10%	Retracted globes within orbits
10% - 12%	Persistent skin tent, dull corneas, and evidence of hypovolemia
> 12%	Hypovolemic shock, death

Inclusion criteria for the Healthy group consisted of no changes in the physical and analytical exams. Animals with concurrent diseases that could influence the PCT serum values were excluded, such as neoplasia, end-stage renal diseases, and acute liver failure.

2.2 Sample Collection and Assessment

The blood samples were collected from the jugular vein at a volume of 2 mL per sample, for diagnostic purposes in the Septic group and for preoperative analysis in the Healthy group.

Following collection, the samples were centrifuged for approximately 15 minutes, and the subsequently separated serum was stored at -20ºC for a maximum of 3 weeks, until it was transferred to the -80ºC freezer located in the Clinical Analysis Laboratory of Lusófona University.

All samples were analyzed in June 2025, at the Clinical Analysis Laboratory of the Faculty of Veterinary Medicine - Lusófona University, using an enzyme-linked immunosorbent assay (ELISA) method, specific for canine pro-calcitonin (BioVendor, Asheville, NC), previously validated for use in dogs (Battaglia *et al*., 2020; Goggs *et al*., 2018).

To perform the assay, standards were first prepared to later construct the standard curve, along with quality controls, wash buffers, antibody solution, and test samples, which were diluted at a ratio of 1:5. The procedure involved incubating all reagents for 1 hour at 25 ± 2 °C while shaking at 100 rpm, rather than the 300 rpm recommended by the manufacturer, as clinicians deemed the higher speed unnecessary. After the addition of the primary antibody, the wells were incubated for another hour. The addition of the antibody conjugate and a further 30 minute incubation followed this. Each incubation step was interspersed with washing steps to ensure proper removal of reagents. Following the final wash, substrate solution was added, and the plate was incubated in the dark for 15 minutes. Finally, a STOP solution was added, and the absorbance of each well was measured using a microtiter plate reader capable of reading at 450 nm or 450/630 nm, with software used for data analysis.

2.3 Statistical Analysis

Statistical analysis was performed using the Jamovi software, version 2.5.3. All results were expressed as mean ± standard deviation (SD). The normality of the data was assessed using the Shapiro-Wilk test. Differences between groups for leukocytes, neutrophils, lymphocytes, platelets, and PCT levels were evaluated with a Student's t-test or the Mann-Whitney rank sum test, as appropriate. Correlations between variables were obtained using Pearson's correlation or Spearman's correlation, as appropriate.

Binary logistic regression analysis was used to assess the association between PCT serum levels and the risk of developing sepsis. Significance was set at $p < 0.05$.

3. Results

A total of 51 dogs were included in this study, recruited from owners who agreed to participate. Of these, 26 were admitted based on the sepsis criterion (sepsis group), and 25 were healthy dogs (healthy group).

In the sepsis group, 7 dogs were male (26.92%) and 19 were female (73.08%). The breed distribution included 7 Cross Breed (26.92%), 4 Labrador Retriever (15.38%), 3 Golden Retrievers (11.54%), 3 German Sheperd (11.54%), 2 Teckel (7.69%), 2 Jack Russel Terrier (7.69%), 1 Cavalier King Charles (3.85%), 1 Setter Gordon (3.85%), 1 French Bulldog (3.85%), 1 Chow Chow (3.85%), and 1 German Spitz (3.85%). The mean age was of 9.62 ± 3.34 years.

In the healthy group, there were 3 male dogs (12%) and 22 female (88%). The breeds included 6 Cross Breed (24%), 4 Labrador Retriever (16%), 2 Miniature Schnauzer (8%), 2 French Bulldog (8%), 2 German Sheperd (8%), 1 Welsh Terrier (4%), 1 West Highland White Terrier (4%), 1 Portuguese Mastiff (4%), 1 Portuguese Water Dog (4%), 1 Irish Setter (4%), 1 Chihuahua (4%), 1 Jack Russel Terrier (4%), 1 Teckel (4%) and 1 Epagneul Breton (4%). The mean age was of 1.85 ± 1.98 years. Among these 25 dogs, 11 (44%) underwent Laparoscopic Ovariectomy, 11 (44%) had Ovariohysterectomy, and 3 (12%) had Orchiectomy.

In the septic group, 8 dogs were diagnosed with pyometra (30.77%), with one of them also having spleen torsion (3.85%). Seven had hemorrhagic gastroenteritis (26.92%), while 6 experienced gastrointestinal perforation or rupture (23.08%). Among those, one also suffered from necrotic pancreatitis (3.85%). Additionally, 2 dogs had severe dog bite wounds (7.69%), 1 had a septic peritonitis (3.85%), 1 was diagnosed with a gastrointestinal linear foreign body (3.85%), and another dog had an open fracture (3.85%).

In the septic group, 16 dogs had a positive outcome (61.5%) and 10 had a negative outcome (38.5%).

Dogs were selected based on a combination of systemic infection criteria to be included in the septic group, which encompassed clinical, imaging, and analytical findings. As the blood cell data will be discussed later, the remaining parameters are presented in Table 6.

Table 6 – Clinical, imaging, and analytical findings that were reported in the included cases that were suggestive of sepsis.

Findings suggestive of sepsis	% of dogs
Prostration	69.2%
Hypoglicemia	46.2%
Peritoneal reactivity on ultrasound	46.2%
Free fluid on ultrasound	42.3%
Dehydration	30.8%
Hematochezia	30.8%
Congestive mucous membranes	26.9%
Pale mucous membranes	26.9%
Tachypnea	19.2%
Hypotension	19.2%
Azotemia	19.2%
Hypothermia	15.4%
Hyperthermia	15.4%
Free gas on ultrasound	11.5%
Tachycardia	7.7%
Hyperbilirubinemia	7.7%
Hematemesis	7.7%
Hypoalbuminemia	3.8%
Free fluid glucose < 20 mg/dL compared to blood glucose	3.8%

Regarding age mean ± SD, significant differences were observed between healthy (1.68 ± 1.90) and septic dogs (9.62 ± 3.41) ($p<0.001$).

Regarding body weight mean ± SD, no significant differences were observed between healthy (15.5± 11.2) and septic dogs (20.8± 12,4) ($p=0.118$).

Regarding body score median, no significant differences were observed between healthy (5) and septic dogs (5) either ($p<0.403$).

The leukocytes were within the normal range (5.05-16.76 K/µL) in 12 dogs (46.15%) of the septic group, 8 dogs had leukocytosis (30.77%), 4 had leukopenia (15.38%), and 2 dogs' results were missing (7.70%). Of those dogs, 5 (19.23%) had neutrophils within the normal range (2.95-11.64 K/µL), 10 had neutrophilia (38.46%), 10 had neutropenia (38.46%) and 1 dog's results were missing (3.85%).

Of the dogs that constituted the healthy group, 22 had leukocytes within the normal range (88%), 1 had leukocytosis (4%) and 2 dogs' results were missing (8%). Twenty-three of the dogs had normal neutrophils (92%) and two dog's results were missing (8%).

The leukocytes mean ± SD showed no significant differences between healthy (11.06 ± 2.32) and septic dogs (18.5 ± 20) (p= 0.632) (Figure 4).

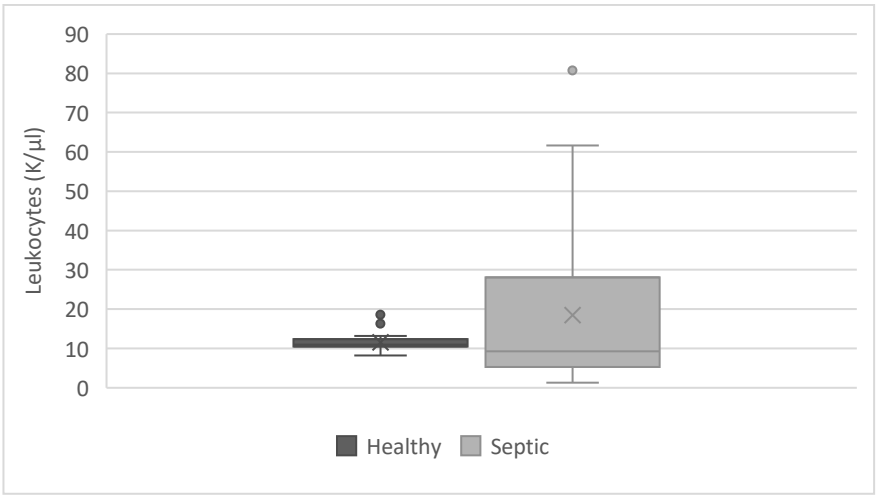


Figure 4 – Leukocytes (K/µL) values in healthy (dark grey) vs septic dogs (light grey). Some data fall outside the normal range (outliers).

The neutrophils mean ± SD showed no significant differences as well between healthy (6.80 ± 1.40) and septic dogs (10.9 ± 11.3) (p= 0.084) (Figure 5).

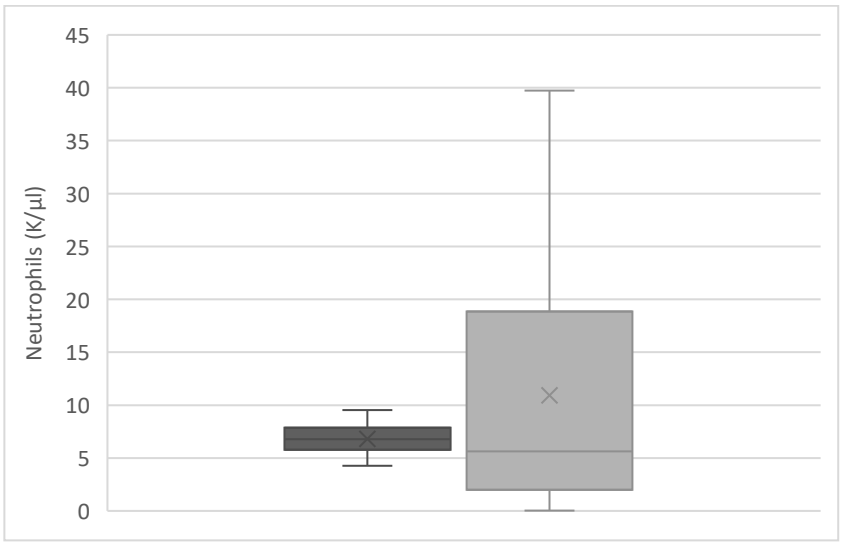


Figure 5 – Neutrophils (K/µL) values in healthy (dark grey) vs septic dogs (light grey).

Regarding lymphocytes mean ± SD, significant differences were observed between healthy (3.39 ± 1.52) and septic dogs (3.27 ± 4) (p= 0.016) (Figure 6).

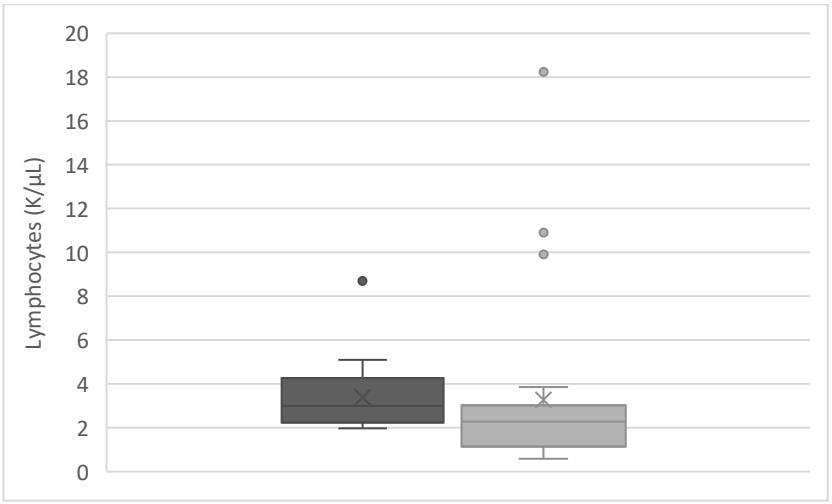


Figure 6 – Lymphocytes (K/µL) values in healthy (dark grey) vs septic dogs (light grey). Some data fall outside the normal range (outliers).

Regarding platelets mean ± SD, no significant differences were observed between healthy (264 ± 58.3) and septic dogs (253 ± 113) (p= 0.316) (Figure 7).

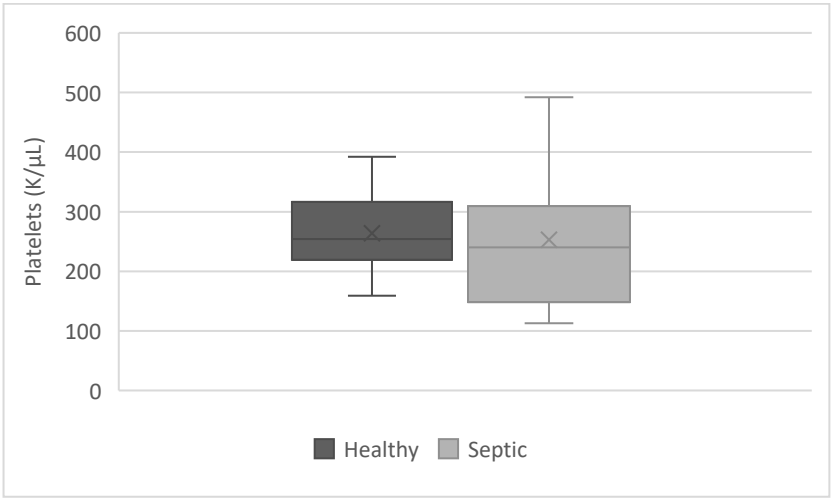


Figure 7 – Platelets (K/µL) values in healthy (dark grey) vs septic dogs (light grey).

PCT mean ± SD showed significant differences between healthy (1.28 ± 0.527) and septic dogs (1.98 ± 1.19) ($p<0.022$) (Figure 8).

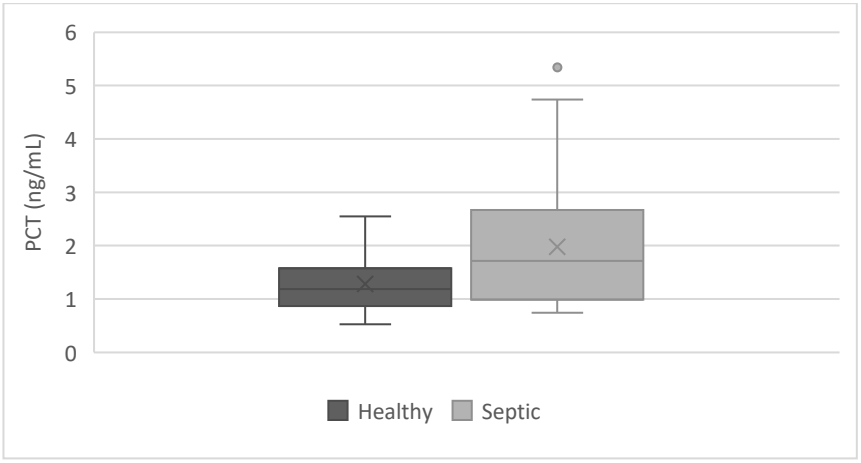


Figure 8 – PCT serum levels in healthy (dark grey) vs septic dogs (light grey). Some data fall outside the normal range (outliers).

With the values that previous studies reported of PCT in healthy dogs, the weighted mean and pooled standard deviation were calculated, and a reference range of PCT serum levels of 0.472 ng/mL ± 0.23 was obtained.

Binary logistic regression was used to evaluate the PCT's ability to predict sepsis. The biomarker was found to be independently associated with sepsis (OR: 2.829; CI 95%: 1.1727-6.824; P=0.021).

Regarding correlations between PCT and blood cells, only a moderate negative linear relationship was observed between PCT and platelets (Table 7).

Table 7 – Correlation (*r*) between Procalcitonin (PCT) and selected hematological parameters.

Correlation	*r*	p-value
PCT-Leukocytes	0.011	0.943
PCT-Neutrophils	-0.049	0.740
PCT-Lymphocytes	-0.112	0.455
PCT-Platelets	-0.355	0.014*

Correlations calculated using Pearson's or Spearman's method. $p < 0.05$ is considered statistically significant.

4. Discussion

Sepsis is a life-threatening organ dysfunction with major physiological and biochemical abnormalities that is one of the main causes of death worldwide, both in human and in veterinary medicine (Cortellini *et al.,* 2024; Rudd *et al*., 2020).

Sepsis has an important health impact, and leads to significant costs for hospitals. A study in human medicine by Canobbio *et al*. (2024) examined the economic impact of sepsis in the largest countries of Europe and found that the cost of sepsis exceeded €2.748 million. The study also revealed that 14% of sepsis cases were diagnosed late, which increased the risk of death.

In people, early diagnosis and subsequent therapeutic intervention are crucial for sepsis management (Cortellini *et al*., 2024). In veterinary medicine, equivalent evidence is missing, but delayed diagnosis likely contributes to worsened outcomes in animals as well. A gold standard diagnostic test for sepsis in dogs still doesn't exist, and there is still no sufficient research that sustains the use of specific exams to identify septic dogs (Cortellini *et al*., 2024).

Because of its global impact, it is urgent to further define sepsis and to acquire more specific diagnostic tools. In human medicine, biomarkers have been widely used in an attempt to accurately diagnose and evaluate the prognosis of sepsis (Pierrakos *et al*., 2020). Although there are no reliable biomarkers for sepsis in veterinary medicine, the behaviour of different biomarkers is an increasingly studied subject than can still be useful.

PCT is a well-known sepsis biomarker in human medicine, that has been reported to be more specific to bacterial infections (Goggs, 2021). In human medicine, PCT is mainly used for the diagnosis of sepsis, attribution of prognosis, and to monitor treatment efficacy (Chivate *et al*., 2016; Meisner, 2014). In veterinary medicine, the studies on PCT are still very limited. Therefore, this study focuses on the potential use of PCT as a diagnostic biomarker for sepsis in dogs. The blood cells were also studied, and an attempt to correlate PCT with blood cells in sepsis was made, to assess if any relation could be observed.

The lack of significant difference between healthy and septic dogs' leukocytes and neutrophils can be explained by the fact that the values of leukocytes and neutrophils of septic dogs have an extremely high SD, meaning that the data have a lot of variability. This is because sepsis can manifest with either leukocytosis or leukopenia, and neutrophilia or neutropenia (de

Laforcade, 2010). Additionally, various septic dogs exhibited extreme values at both ends of the spectrum, further contributing to the wide value range.

Some of the dogs with sepsis had normal leukocytes and neutrophils, which can happen in early stages of sepsis. Moreover, the values don't take into account the evolution of the parameters, since some of the animals had a significant increase or decrease of leukocytes and neutrophils, but not enough to go off the reference range.

The difference that was observed between lymphocytes of septic and healthy dogs can be attributed to the extensive lymphocyte apoptosis that occurs during sepsis (Wang *et al*., 2024). In this context, the neutrophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio, both associated with lymphopenia, have been investigated in veterinary medicine to study its potential to predict mortality in cases of sepsis and/or SIRS (Pierini *et al*., 2019, 2020). Although there has been some contradictory evidence, the study by Pierini *et al.* (2020) showed that the platelet-to-lymphocyte ratio was associated with a 50.5% increase risk of death in dogs. Therefore, although evidence is still lacking, the results of this study and of Pierini *et al*. (2020) underscore the possible clinical relevance of lymphopenia and of its derived ratios as potential prognostic biomarkers of sepsis in dogs.

The lack of significant difference between platelets of healthy and septic dogs may be because both thrombocytopenia and thrombocytosis may occur during sepsis, leading to a high SD (Llewellyn *et al*., 2017; Pierini *et al*., 2020).

Regarding PCT, the results of this study support the hypothesis that serum PCT concentrations differ between healthy and septic dogs - the PCT mean ± SD of healthy dogs is 1.28 ± 0.527, whereas in septic dogs it is 1.98 ± 1.19, with a $p<0.022$.

A reference range for PCT levels in healthy dogs was established at 0.472 ng/mL ± 0.23. This range was calculated with the serum PCT values measured in the present study, combined with the results from previous studies that used the same ELISA kit. While additional research is needed to further validate this reference range, it may serve as a guideline value for future investigations.

This study also reinforces the existence of an association between elevated PCT levels and the occurrence of sepsis, suggesting that PCT might serve as a diagnostic marker for sepsis in dogs.

These results align with those observed in previous studies, which indicate that PCT values are higher in dogs with severe systemic infections, compared to healthy dogs (Battaglia *et al*., 2020; Easley *et al*., 2020; Goggs *et al*., 2018; Troia *et al*., 2018). However, the inexistence of sufficient research that sustains the use of specific exams to identify sepsis in dogs, might bias results in every sepsis study, since the enrollment of animals with less severe disease or even without sepsis can interfere with the results (Cortellini *et al*., 2024). Therefore, it is important to address every case included in this study as sepsis or suspicion of.

Firstly, it is important to remind that, currently, the diagnosis of sepsis in veterinary medicine remains closely associated with SIRS, where a presumptive diagnosis of sepsis typically requires the presence of ≥ 2 SIRS criteria in conjunction with a confirmed or strongly suspected infection (Sharp, 2019). Unlike human medicine - where the definition of sepsis was revised by Singer et al. (2016) - veterinary medicine has yet to adopt validated, species-specific criteria. Therefore, this study included animals if there was confirmed or strong suspicion of infection, ≥ 2 SIRS criteria, and the animal presented clinical or analytical signs associated with the hyper- and hypodynamic phases that occur in sepsis/SIRS (Silverstein, 2014).

Pyometra was the primary diagnosis in the animals included in the sepsis group. While not all cases of pyometra progress to sepsis, the condition has been associated with sepsis in up to 60% of female dogs (Hagman, 2018). In this study, all dogs diagnosed with pyometra exhibited clinical and laboratory signs suggestive of sepsis—such as hypoglycemia, pyrexia, altered mental status, hypotension, tachypnea, leukocytosis, leukopenia, neutrophilia, neutropenia, or elevated band neutrophil counts. Additionally, one of the dogs initially diagnosed with pyometra was later diagnosed with acute splenic torsion, which is described to be possibly associated with the development of sepsis (Niles, 2015).

The second most common diagnosis in the sepsis group was hemorrhagic gastroenteritis. While hemorrhagic diarrhea can increase the risk of bacterial translocation and subsequent sepsis, not all cases lead to systemic infection (Dupont *et al*., 2021). Therefore, for inclusion in the sepsis group, animals presenting with hemorrhagic diarrhea also needed to exhibit additional systemic signs such as hyperemic or pale mucous membranes, altered mental status, pyrexia, hypoglycemia, tachycardia, leukocytosis, leukopenia, neutrophilia, neutropenia, or elevated band neutrophils.

Gastrointestinal leakage, such as perforation or rupture, is the most frequent cause of septic peritonitis in small animals, which causes intraperitoneal infection by commensal

intestinal bacteria (Dayer *et al*., 2013). In this study, the animals diagnosed with these conditions were immediately included in the sepsis group, due to the presence of an obvious systemic bacterial infection. However, they all also had various clinical and analytical signs related to sepsis, such as hyperemic mucous membranes, pale mucous membranes, pyrexia, hypothermia, altered mental status, hypoglycemia, tachycardia, tachypnea, hypotension, leukocytosis, leukopenia, neutrofilia, neutropenia or band neutrophil concentrations.

One of the dogs with gastrointestinal rupture also had necrotizing pancreatitis, and one of the major complications of this condition is septic peritonitis and sepsis (Cleveland Clinic, 2023; Judge, n.d.).

Both cases involving dogs severely attacked by other dogs were immediately classified as suspected sepsis as well. This classification was based on the severity of the injuries, characterized by multiple wounds, and the well-documented risk of infection associated with dog bites, since multiple bacteria can be isolated from dog bites (Son & Song, 2024). In addition to the high risk of infection, both dogs exhibited clinical signs consistent with SIRS and sepsis, including tachycardia, tachypnea, hypotension, leukopenia, neutropenia, hypoglycemia, altered mental status, pyrexia, or hypothermia. Neither animal showed signs of profuse bleeding or severe dehydration.

The dog with septic peritonitis had very nonspecific signs, such as pyrexia, hypoglycemia, severe diarrhea, and vomits. On ultrasound, an abdominal effusion and generalized peritonitis were observed, and, after cytology, the effusion was identified as septic exudate, the reason why it was then included in the sepsis group.

The dog diagnosed with a gastrointestinal linear foreign body was included in the sepsis group since, after surgical removal of the foreign body, he developed clinical signs compatible with sepsis, such as hypoglycemia, altered mental status, pyrexia, tachypnea, leukocytosis, and neutrophilia.

Fracture-related infection is a major complication of open fracture wounds, happening because there is exposure of bone to the environment (Perry, 2016). The disruption of soft-tissue integrity increases the risk of infection as well. One of the dogs included in the Sepsis group had two open fractures with extensive soft tissue laceration. Furthermore, the dog had clinical signs compatible with systemic bacterial infection, such as pyrexia, tachypnea, leukopenia and neutropenia.

A few dogs in the sepsis group had previously diagnosed concomitant diseases, including hyperadrenocorticism, benign prostatic hyperplasia, diabetes mellitus, hypothyroidism, spleen and liver lymphoma, exocrine pancreatic insufficiency, and chronic kidney disease.

As mentioned earlier, research on PCT in veterinary medicine remains limited. However, given that PCT appears to behave similarly in dogs and humans, certain hypotheses from human medicine may be carefully extrapolated, though no conclusions can be drawn.

In human medicine, a potential association between elevated PCT levels and prostate cancer has been reported, but no such correlation has been found with benign prostatic hyperplasia (Ilktac *et al*., 2021).

Regarding diabetes mellitus, high PCT levels are not directly linked to the disease itself. However, a correlation has been identified between PCT and diabetic ketoacidosis, likely due to the presence of underlying infections—a known trigger for ketoacidosis (Thiab *et al*., 2023). Nevertheless, PCT appears to reflect the presence of infection rather than ketoacidosis per se. Importantly, the diabetic dog in this study showed no signs of diabetic ketoacidosis, and PCT has not been associated with uncomplicated diabetes mellitus.

Chronic kidney disease has recently been associated with higher PCT levels, which might interfere with the interpretation of the results (Wu *et al*., 2019). However, it was described that the PCT levels are directly correlated with the stage of the chronic kidney disease, meaning that the higher the stage, the higher the PCT values. The dogs that participated in this study presented an IRIS 2 and early IRIS 3 stage, and although the studies in humans don't use this staging system, it is relevant to take this into account (IRIS, 2023).

Conversely, conditions such as hyperadrenocorticism, hypothyroidism, lymphoma, and exocrine pancreatic insufficiency have not been reported to affect PCT levels (Piperidou *et al*., 2022).

Finally, an attempt was made to investigate the association between PCT levels and blood cell parameters. The only significant correlation observed was a moderate negative linear relationship with platelet count, indicating that, when one of the variables increases, the other tends to decrease. This result comes in agreement with a human medicine study by Chen *et al*. (2023), which also observed a negative correlation between PCT level and platelet counts in septic patients.

Although both thrombocytopenia and thrombocytosis can occur in sepsis, thrombocytopenia has been linked with higher mortality rates, and high PCT levels are also associated with a worse prognosis (Al Saleh & AlQahtani, 2021; Anand *et al*., 2013; Chen *et al*., 2023). Therefore, this relation might be valuable to predict mortality risk in septic patients, if additional investigations are conducted both in human and veterinary medicine.

This study has various limitations. Firstly, we studied a heterogenous sample of dogs with sepsis, with various sources of infection and with sepsis caused by a range of different organisms. Similarly, the dogs with sepsis enrolled in our study were not enrolled at the same stage of their disease processes and were also affected by varying degrees of their disease process.

Another important limitation of this study is that all sepsis diagnoses were presumptive, because no blood cultures were conducted. Therefore, even with justifying the inclusion of each case, there isn't any certainty of the diagnosis performed. The bacterial load and illness severity weren't approached, which could bias the results as well, especially since different emergencies were included in this study. A way around these limitations would be to perform blood cultures to animals included in future sepsis studies, not only to diagnose but to access the bacterial load, and to use scores that would indicate illness severity, such as SOFA or qSOFA score, and/or the Canine Acute Physiologic and Laboratory Evaluation Score (APPLE score) or APPLE fast score (Judge, 2023).

Lastly, another limitation in this study, is that the collected samples might have suffered alterations due to the delay in storage in the -80ºC freezer, although it is not described that this can alter PCT values.

5. Conclusion

This study helped to establish a reference range for serum PCT levels in healthy dogs, by integrating data from previous research, proposing the value of 0.472 ± 0.23 ng/mL.

The findings also reinforce the potential role of PCT as a diagnostic biomarker for sepsis in canine patients, as elevated levels were significantly associated with sepsis.

Furthermore, the investigation into hematological correlations revealed a moderate negative linear relationship between PCT concentration and platelet count. This association is also reported in human medicine, where thrombocytopenia and high PCT levels are both linked to poorer prognoses in septic patients. While the current results do not establish a causal relationship, they suggest that a combined assessment of PCT and platelet count could provide valuable prognostic insight and aid in mortality risk prediction.

Overall, this study contributes to the increasing evidence that supports PCT's diagnostic and prognostic utility in veterinary medicine. Future research, incorporating larger sample size and applying different methods, is necessary to help validate these findings, in order to further assess their applicability in clinical practice.

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