UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

CANINE PARVOVIRUS AND SEPSIS: SIRS CRITERIA EVALUATION AND IMPLEMMENTATION OF A PIRO CLASSIFICATION

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Resumo

Parvovirose Canina e Sépsis: Avaliação dos critérios de SIRS e Implementação da classificação PIRO

A sépsis esta associada a uma elevada prevalência e taxa de mortalidade**.** A Parvovirose canina predispõe para o aparecimento de sépsis secundaria à translocação bacteriana intestinal e imunossupressão. Este facto faz dos cães naturalmente infetados com parvovírus uma boa população para o estudo de sépsis. O principal objetivo deste estudo foi avaliar as diferenças entre dois conjuntos de critérios de SRIS (Síndrome de Resposta Inflamatória Sistémica) sobre a sua capacidade de prognóstico, assim como avaliar a possibilidade de implementação de um sistema de estratificação de animais sépticos com base no modelo PIRO (Predisposition, Infection, Response, Organ dysfunction). Os 72 animais da amostra foram submetidos a dois conjuntos de critérios SIRS e classificados para cada um dos elementos constituintes do PIRO (com exceção da infeção, sendo que todos os animais foram considerados como tendo a mesma classificação para a Infeção), avaliando a sua relação com o desfecho. Os dados foram recolhidos a partir dos registos clínicos da Unidade de Isolamento de Doenças Infeciosas (UIDI) do HEV-FMV-UL.

Em relação aos critérios de classificação SRIS, os resultados revelaram que a alteração proposta aos critérios originais resulta numa associação estatisticamente significativa com o desfecho (OR = 4.09, $p < 0,05$), contrastando com os resultados observados quando aplicados os critérios originais ($p=0.352$) que não se correlacionam significativamente com o desfecho. Não foi encontrada nenhuma associação estatisticamente significativa entre a Predisposição ($p=1$), Resposta ($p=0.1135$), Disfunção Orgânica ($p=0.1135$) ou PIRO total ($p=0.093$) e o desfecho clínico. Os resultados obtidos revelam a necessidade de critérios mais específicos para a avaliar SRIS e sépsis. Os resultados sugerem que o aumento da especificidade pode melhorar o seu valor prognóstico.

Este trabalho representa uma contribuição para o desenvolvimento de um conjunto de critérios consensual e aprovado para a classificação de animais sépticos, servindo de base para estudos futuros. Mais critérios com uma maior especificidade, como marcadores bioquímicos inflamatórios e de disfunção orgânica, devem ser adicionados ao sistema PIRO proposto.

Estudos futuros devem concentrar-se em melhorar os sistemas de classificação existentes e descobrir novos biomarcadores que permitam uma intervenção atempada em animais afetados por sépsis, melhorando a taxa de sobrevivência.

Palavras-chave: Sepsis, Parvovirus canino, SRIS, PIRO, UIDI

Abstract

Canine Parvovirus and Sepsis: SIRS criteria evaluation and implementation of a PIRO classification

Sepsis is a severe condition associated with high prevalence and mortality rates. Parvovirus enteritis is a predisposing factor for sepsis, as it promotes intestinal bacterial translocation and severe immunosuppression. This makes naturally parvovirus infected dogs a suitable study population as far as sepsis is concerned. The main objective of the present study was to evaluate the differences between two sets of SIRS (Systemic Inflammatory Response Syndrome) criteria in outcome prediction, parallelly the possibility of stratifying and classify septic animals using a proposed animal adapted PIRO (Predisposition, Infection, Response, Organ dysfunction) scoring system was also assessed. The 72 animals enrolled in this study were subjected to a score for each of the PIRO elements (except for the Infection, as all were considered to have the same infection score) and to two sets of SIRS criteria, assessing their correlation with the outcome. The data was retrieved from the clinical records of the Infectious Disease Isolation Unit (IDIU) of the Veterinary Teaching Hospital (VTH) of the Faculty of Veterinary Medicine (FMV) of the University of Lisbon (ULisboa).

Concerning the SIRS criteria, it was found that the proposed alterations were significantly associated with the outcome (OR = 4.09, $p < 0,05$), contrasting with the original SIRS criteria ($p=0.352$) that did not correlate with the outcome. No significant statistical association was found between Predisposition ($p=1$), Response ($p=0.1135$), Organ dysfunction ($p=0.1135$) or total PIRO score ($p=0.093$) and outcome. The results obtained reveal the need for consensual and more specific criteria to assess SIRS and sepsis. The results suggest that augmenting the criteria specificity may improve their prognostic value, thus making them more useful in clinical management and treatment decision.

This work represents a contribution for the development of an approved set of criteria, to could contribute not only to the classification of septic dogs but also to the improvement of sepsis diagnosis. Further studies are still needed to conclude about the best criteria to be used, but this study can serve as base from which further studies can adapt and improve. Additional more specific criteria, mainly inflammatory and organ dysfunction biomarkers, should be added to the proposed PIRO scoring system in order to improve the its´ prognostic value and clinical utility.

Further studies should focus on improving classification systems and finding new biomarkers that would allow a timely intervention in sepsis affected animals and improve sepsis survival rate.

Keywords: Sepsis, Canine parvovirus, SIRS, PIRO, IDIU

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Abbreviation List:

AKI – Acute Kidney Injury

ALP – Alkaline Phosphatase

ALT – Alanine Transaminase

AP-1 – Activator Protein 1

APACHE – Acute Physiology and Chronic Health Evaluation

APPs – Acute Phase Proteins

ARDS – Acute Respiratory Distress Syndrome

ARF – Acute Renal Failure

AUC-ROC – Area Under the Receiver Operating Characteristics Curve

bpm – beats per minute

ºC – degrees Celsius

CIRCI – Critical Illness Related Corticosteroid Insufficiency

CPV – Canine Parvovirus

COX-2 – Cyclo-oxygenase

CRP – C-Reactive Protein

CRT – Capillary Refill Time

DAMPs – Damage Associated Molecular Patterns

DD – D-dimer

DIC – Disseminated Intravascular Coagulation

FDP – Fibrin degradation Products

FFT – Fast-and-Frugal Tree

FNA – Fine Needle Aspiration

GI – Gastrointestinal

HEV-FMV-UTL – Veterinary Teaching Hospital of the Faculty of Veterinary Medicine of the University of Lisbon

HK – High Molecular Weight Kininogen

HR – Heart Rate

ICU – Intensive Care Unit

IDIU – Infectious Disease Isolation Unit

IL – Interleukin

IRAP-1 – Interleukin 1 Receptor Antagonist Protein

iNOS – Inducible Nitric Oxide Synthase

IV – Intravenous

LPS – Lipopolysaccharide

MDA – Maternally Derived Antibodies

MEDS – Mortality in Emergency Department Sepsis

mmHg – millimetre of mercury

MODS – Multiple Organ Dysfunction Syndrome

NF-κB – Nuclear Factor Kappa B

NO – Nitric Oxide

OR – Odds Ratio

PAI-1 – Plasminogen Activator Inhibitor Type 1

PAMPs – Pathogen Associated Molecular Patterns

PCO2 – Partial Pressure of Carbon Dioxide

PIRO – Predisposition, Insult, Response, Organ Dysfunction

PK – Prekallikrein

PRRs – Pattern-Recognition Receptors

PT – Prothrombin Time

PTT – Partial Thromboplastin Time

qSOFA – quick SOFA

RR – Respiratory Rate

SIRS – Systemic Inflammatory Response Syndrome

SOFA – Sequential/ Sepsis-related Organ Failure Assessment

sTREM – soluble Triggering Receptor Expressed on Myeloid Cells

T – Temperature

TF – Tissue Factor

TNF- α – Tumour Necrosis Factor-α

TNFR – Tumour Necrosis Factor Receptor

UIDI – Unidade de Isolamento de Doenças Infeciosas

WBC – White Blood Cells

µL – microliter

I - Activities developed during the curricular internship

This report concerns the curricular internship that occurred between 10th September 2018 and 11th January 2019 at VetOeiras - Central Cascais Veterinary Hospital and between 14th January 2019 and 15th March 2019 at the Isolation Unit for Infectious Disease, University of Lisbon's Faculty of Veterinary Medicine. The activities developed during the internship encompassed different clinical areas including surgery, internal medicine, and inpatient care with a rotating rota trough them with a total number of hours of 1264 (320h internal medicine, 300h surgery, 300h inpatient care and 344h in the Isolation Unit for Infectious Disease). The rotation included different working periods with day, night and weekend shifts that varied from 8 to 15 working hours a day. All the activities took place under the supervision of the Veterinary Surgeon or Veterinary Nurse on duty.

1. Internal Medicine

During the time spent in the internal medicine service there was opportunity to attend first time, revaluation and reference appointments during which it was possible to participate in the collection of prior clinical history and anamnesis of the patients as well as a thorough physical exam to each one of them. Under the supervision of the Veterinary Surgeon there was also the opportunity to participate in some medical procedures such as peripheral intravenous catheterization, blood samples analyses (hemogram and biochemical analyses), imagiological complementary exam (ultrasonography, radiography and endoscopy), to perform cytological evaluation (obtained via FNA or swab) and preparation and administration of medication. There was also the chance to discuss, with the responsible clinician, the different possible approaches, differential diagnosis and treatment options to each of the clinical cases.

2. Surgery

The scheduled surgeries took place from Monday to Friday morning, with emergency surgery occurring whenever needed. The activities developed in the surgery department of VetOeiras included reception of the animals and preoperative hemogram and biochemical screening, peripheral venous catheterization, preanesthetic drugs preparation and administration, trichotomy and surgical asepsis, anaesthetic induction and monitoring, assistance in the surgical procedures, and post-surgical monitorization. The surgeries witnessed fell mainly under the Orthopaedic and Soft Tissue surgery field with the opportunity to observe and participate in some Ophthalmological surgery and Neurosurgery.

3. Inpatient Care

During the hours spent in the inpatient care unit of VetOeiras there was the chance to follow the progress of the different clinical cases, participating on the animals clinical monitoring, feeding plan, and drugs preparation and administration according to the indications of the Veterinary Surgeon responsible for the case. There was also the opportunity to discuss not only the evolution of the animals' condition with the clinician responsible, as well as the discharge plan for each one. It was also possible to participate in some medical procedures including wound cleaning and dressing, blood sample collection and analyses, peripheral venous catheterization and urethral catheterization. Some of the cases offered the opportunity to participate in critical care treatments and monitoring and to assist in some emergency procedures such as cardiorespiratory resuscitation.

4. Infectious Diseases Isolation Unit

The activities developed in the Infectious Diseases Isolation Unit (IDIU) of the Faculty of Veterinary Medicine were fundamental in allowing the accomplishment of the present work since it gave the opportunity to accompany some Canine Parvovirus Infections, allowing the characterization of its´ clinical presentation. The activities that took place included clinical monitoring, medication preparation and administration and the correct hygienization of contaminated infectious material. All the activities took place under the supervision of Professor Solange Gil. It was possible to assist in some infectious disease consultations and to participate in the clinical discussion concerning the differential diagnosis and treatment options. The possibility to access the Unit´s clinical record allowed to obtain the data needed to accomplish the present Master Thesis.

II – Literature Review

1. Sepsis

1.1. General notions and SIRS:

The concept of sepsis has undergone a series of alterations since it has first been associated with bacterial infection approximately 100 years ago (Vincent et al. 2011). As it stands, according to the most recent scientific consensus, the term sepsis should be used to describe the organ dysfunction triggered by a deleterious inflammatory host response to infection (Singer et al. 2016). Despite being the most common cause of sepsis, bacteraemia is not a mandatory condition for sepsis to occur (Greiner et al. 2008). It is also important to note that bacteraemia can be present in non-septic animals. These

animals present positive blood cultures, and therefore viable bacteria in their bloodstream, but do not show any signs of inflammatory response. After the release of bacteria into the bloodstream as a result of an infection (abdominal and respiratory tract infections being the most common sepsis inducing for dogs, and septic peritonitis, pyothorax, and hepatic abscessation for cats (Ettinger et al. 2017)), sepsis only takes place if the host immune system is overpowered resulting in clinically significant bacteraemia. The most common organisms found in the bloodstream of dogs with bacteraemia are Staphylococcus spp. (including S. pseudintermedius, S. aureus, and coagulase-negative staphylococci), Streptococcus spp. (particularly S. canis, but also S. bovis complex organisms and seldom other streptococci) and Escherichia coli (Sykes, 2014a).

A related concept is endotoxemia which refers to the presence of lipopolysaccharide (LPS) of the Gram-negative bacterial cell wall in the bloodstream, without necessarily being associated with the presence of live bacteria (Silverstein & Otto, 2012).

To clarify the correlation between the systemic inflammatory response and sepsis a conference was held in 1991, from which arose the criteria to assess if a systemic inflammatory response syndrome is taking place in humans. Since the first adaptation of these criteria for animals (Purvis and Kirby 1994) they have been subjected to a series of alterations and the cut off values slightly vary among authors. On behalf of augmenting the sensitivity and specificity of the diagnosing capability of these parameters, it is important to use them in association with the clinical judgement when screening animals for sepsis, as they are not sufficiently accurate to establish a definitive diagnosis (Hauptman et al. 1997).

Table 1: SIRS diagnosing criteria for cat and dog. (Sykes, 2014a)

Criteria	Dog	Cat
Temperature	<37.8 or >39.4 ºC	<37.8 or >39.7 ºC
HR	>140 beats/min	<140 or >225 beats/min
RR	>30 breaths/min or PCO_2 < 32 mmHg	>40 breaths/min
Leukocyte count	<6000 or >16,000 cells/µL, or >3% band neutrophils	19,500 cells/µL, or >5% band neutrophils

Legend: ºC, degrees Celsius; HR, heart rate; RR, respiratory rate; PCO2, partial pressure of carbon dioxide; mmHg, millimetre of mercury; cells/µL, cells per microliter

For a dog to be diagnosed with SIRS at least 2 of the 4 criteria need to be met: body temperature <37.8 ºC or >39.4 ºC, HR >140 bpm, RR >30 breaths/min or PCO2 < 32 mm Hg (venous or arterial), WBC <6000 or > 16,000 cells/µL, or >3% band neutrophils. Cats need to meet 3 of the 4 criteria for SIRS to be diagnosed: body temperature <37.8 ºC or >39.7 ºC, HR < 140 or >225 bpm, RR > 40 breaths/min, WBC > 19,500 or <5000 cells/µL, or >5% band neutrophils (Sykes 2014a).

As stated at the most recent human medicine consensus the term severe sepsis should no longer be used to describe sepsis-induced organ dysfunction or tissue hypoperfusion, as this description falls under the definition of sepsis. When the circulatory and cellular/metabolic abnormalities are severe enough to increase mortality, septic shock should be considered (Singer et al., 2016). This translates to a sepsis associated hypotension non-responsive to intravascular volume expansion, i.e., dogs with systolic blood pressure < 90 mm Hg or mean arterial pressure < 70 mm Hg that only respond to vasopressor therapy (Sykes, 2014a).

1.2. Pathogenesis

1.2.1. Innate immunity and inflammatory mediators

The instigation of the systemic inflammatory response begins with the activation of the innate immune system cells (mainly macrophages, monocytes, neutrophils, and natural killer cells), either by pathogen associated molecular patterns (PAMPs) or damage associated molecular patterns (DAMPs).

PAMPs are molecules originated from the pathogen invading the animal and can vary from endotoxin from Gram-negative bacteria to exotoxins, peptidoglycans, or superantigens from Gram-positive bacteria or even fungal cell wall material (Gyawali et al. 2019). On the other hand, DAMPs are endogenous molecules or material released by damaged cells as consequence of trauma, ischemia, malignancy, inflammatory diseases or other events capable of causing cellular stress (King et al. 2014).

Both PAMPs and DAMPs can be recognized by the innate immune system by binding to specific receptors denominated pattern-recognition receptors (PRRs). PRRs can be found in various different cells, including dendritic cells, macrophages, B cells, natural killer cells, endothelial cells, epithelial cells, and fibroblasts.(Lewis et al. 2012).

After the recognition of the molecular patterns, an intracellular signalment cascade pathway arises culminating in the activation of nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1), whose function is to enter cell nucleus and activate the transcription sites of multiple genes that codify, among other, acute phase proteins,

coagulation factors and pro-inflammatory cytokines such as tumour necrosis factor (TNF)- α and Interleukins (ILs) 1, 6, 8, and 12 (Lewis et al. 2012).

Macrophages and T cells are the main producers of TNF-α. This molecule is responsible for many of the manifestations of the inflammatory status, as it promotes vasodilation and blood stasis as a result of inducible nitric oxide synthase (iNOS) and cyclo-oxygenase 2 (COX-2) production (Lewis et al. 2012). Another consequence of TNF-α production is neutrophils activation and leukocytes chemotaxis to the endothelial wall, subsequently to the upregulation of endothelial adhesion molecules (King et al. 2014).

1.2.2. Inflammation and acute phase response

Pro-inflammatory cytokines are answerable for the inflammatory clinical manifestations recognised during sepsis with alterations including hyperthermia, tachycardia, leucocytosis, exudation, hypotension, acute phase response and neutrophils chemotaxis to the affected site (Giamarellos-Bourboulis 2010).

ILs 1 and 6 are the principal accountable for the inflammatory response associated with sepsis. IL-1, in its two proinflammatory forms IL-1α and IL-1β, besides being responsible for the endothelial cell adhesion molecules and cytokines production, is also the major endogenous pyrogenic agent and acute phase response stimulator (Silverstein and Otto 2012). IL-6 takes an important role in leukocyte activation and myeloid cell proliferation, despite also being responsible for fever induction and triggering the acute phase response (Lewis et al. 2012).

In addition, consequently to the activation of PRRs, hepatocytes are triggered to release acute phase proteins (APPs) contributing to the animal´s response to the inflammatory stimulus by promoting fever, neutrophilia and activation of coagulation and complement cascades. Type I APPs are induced by IL-1α and IL-1β and TNF-α and type II are induced by IL-6. Even though some APPs, particularly procalcitonin and C-reactive protein (CRP), can be of predictive value when it comes to mortality in sepsis in humans and animal trials, there is still the need to find a better method to predict the outcome for SIRS affected animals (Chan et al. 2009; Lewis et al. 2012).

1.2.3. Anti-inflammatory agents and immunosuppression

Anti-inflammatory mediators are present alongside inflammatory mediators since the onset of sepsis with the animals' state fluctuating between hyperinflammation and hypo-inflammation. The principal cytokine implicated in the anti-inflammatory response is IL-10. This interleukin anti-inflammatory action is a result of TNF-α, IL-1β and IL-6 release inhibition, as well as IL-1 receptor antagonist protein (IRAP-1) production (Lewis

et al. 2012). Tumour necrosis factor receptor (TNFR) 1 and 2 and soluble triggering receptor expressed on myeloid cells (sTREM) 1 also play a role in the anti-inflammatory response (Giamarellos-Bourboulis 2010).

One of the consequences of the anti-inflammatory response is the impairment of the immune system, prompting the host to secondary infections via bacteria, viral or fungal exposure. Alterations during the anti-inflammatory state that contribute to the immunosuppression include: Th2 and Treg response prevalence over Th1 response, CD4+ lymphocytes depletion and overall lymphocytes disfunction and apoptosis, inflammatory cytokines (such as IL-6 and TNF) impaired production and neutrophils reduced phagocytic and chemotaxis aptitude (King et al. 2014; Gyawali et al. 2019).

1.2.4. Inflammation and dysregulated coagulation

Parallelly to the inflammatory response there is a stimulation of the haemostatic system with variable clinical presentation raging from non-symptomatic thrombocytopenia to severe disseminated intravascular coagulation (DIC). During sepsis haemostasis deviates to a hypercoagulability state, with consequent thromboembolic events that contribute to organ failure, this is followed by a hypocoagulability state triggered by fibrinolytic events and by coagulation factors and platelets consumption (King et al. 2014; Gyawali et al. 2019).

Tissue factor (TF) is the main instigator of the coagulation abnormalities that take place during SIRS. TF becomes exposed to the bloodstream after endothelial damage but it´s also produced by cytokine and PAMPs stimulated monocytes, macrophages, parenchymal cells and neutrophils, causing activation of the extrinsic coagulation pathway resulting in thrombin production, platelets activation and fibrin-clot formation (Lewis et al. 2012; King et al. 2014).

In healthy animals anticoagulants counter the hypercoagulability events triggered by TF, thus balancing the coagulation state. During sepsis these counterbalance mechanisms are impaired due to decreased levels of antithrombin and reduced protein C activation. Antithrombin is diminished in sepsis as a result of a decreased hepatic synthesis, consumption subsequently to the formation of thrombin-antithrombin complexes, and deterioration by activated neutrophils elastase release.

Thrombin is responsible for thrombomodulin activation, which is accountable for converting protein C into activated protein C that promotes fibrinolysis and anticoagulation via cleavage of factors Va and VIIIa. In the septic process downregulation of thrombomodulin combined with overconsumption and reduced production of protein C tilts the animal toward hypercoagulability.

Increasing levels of inflammatory cytokines promotes the release of tissue plasminogen activators from endothelial cells with consequential increase of plasmin activation. Counterbalancing mechanisms result in plasminogen activator inhibitor type 1 (PAI-1) increment resulting in decreased fibrin clots degradation that contribute to the hypercoagulability state (King et al. 2014; Gyawali et al. 2019).

It is also relevant to mention the role of the contact phase system. When triggered by polyphosphate, collagen, protein aggregates, LPS, glycosaminoglycans, or nucleic acids, this system consisting of coagulation factors XII and XI, plasma prekallikrein (PK), and nonenzymatic cofactor high molecular weight kininogen (HK), contributes to the altered coagulation. Surface contact promotes factor XII activation into to factor XIIa which is responsible for PK activation and consequent kallikrein release. Kallikrein is then responsible for further activation of factor XII resulting in positive feedback cycle of coagulation stimulation (Lewis et al. 2012; Wu 2015).

1.2.5. Organ dysfunction

The definition of multiple organ dysfunction syndrome (MODS) has remained unchanged since it was defined as “the presence of altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention” in the first sepsis consensus conference in 1991 (Bone et al. 1992). During sepsis MODS can be considered when at least two organ systems, distant to the infection site, became dysfunctional (Sykes 2014a).

The intricate pathophysiology of MODS makes it hard to come to an agreement, with different models being proposed. Different mechanisms act together contributing to the induction of organ failure: hypoxic cellular and tissue damage, apoptosis induction, gastrointestinal microbiota translocation, immune system dysregulation and mitochondrial dysfunction.

Even though all the above contribute to organ failure, immune system dysregulation and mitochondrial dysfunction are the ones thought to have a key role in MODS pathogenesis. Neutrophils stimulated by inflammatory cytokines chemotaxis to different tissues where they produce superoxides responsible not only for tissue damage, but also for the perpetuation of the inflammatory response. Mitochondrial respiration is impaired by the action of superoxide released from neutrophils and nitric oxide (NO) originated from vascular endothelium, this process is denominated cytopathic hypoxia and causes decreased cellular energy production with consequential cellular dysfunction or death (Mizock 2009; Osterbur et al. 2014).

The decrease of oxygen delivery and utilization can have an impact in several organ systems with different clinical presentations. Hypoperfusion secondary to septic

shock is the primary responsible for the hepatic injury verified in sepsis. This first stage of hepatic dysfunction is characterized by cellular damage with aminotransferases increments and diminished gluconeogenesis and glycogenolysis and consequent hypoglycemia. The later stage of hepatic dysfunction results from Kupffer cell activation and release of further inflammatory and oxidative agents, leading to added cellular damage. Parameters that can be used to assess hepatic dysfunction include hyperbilirubinemia, increased alanine transaminase (ALT) or alkaline phosphatase (ALP) and the presence of hepatic encephalopathy. It is important to monitor the hepatic markers as the liver is considered to be the most affected organ in septic dogs (Osterbur et al. 2014; Gyawali et al. 2019).

Respiratory capacity is also affected during sepsis. Animals subjected to a septic insult can develop an acute respiratory distress syndrome (ARDS) characterized by pulmonary edema, structural cellular damage and atelectasis resulting from loss of alveolar-capillary barrier, increased vascular permeability, neutrophil infiltration of the pulmonary tissue and action of proinflammatory cytokines (Wilkins et al. 2007; Osterbur et al. 2014).

The cardiovascular function is affected by both decreased cardiac output and loss of vascular resistance. Inflammatory cytokines promote these cardiovascular changes by increasing vascular permeability and peripheral vasodilation, which contribute to hypovolemia and blood flow variations. Cytokines, endotoxins and calcium changes act together causing downregulation of cardiac contractility. When vasopressor therapy is needed to reverse the resulting hypotension, the animal can be considered to be in septic shock.

The renal impact of sepsis usually leads to acute renal failure (ARF). More commonly, the sepsis associated ARF is triggered by the circulating cytokines, that promote apoptosis with no macroscopic tissue damage. ARF can also come as a consequence of renal hypoperfusion and ischemia, tubular necrosis takes place and glomerular function is impaired.

Gastrointestinal signs of sepsis include vomiting and diarrhoea, ileus and signs of mucosal ulceration like melena and hematochezia. Blood flow alterations, mitochondrial dysfunction and cell apoptosis promoted by cytokines all contribute for the loss of integrity of the intestinal wall, the consequent bacterial translocation can act as a perpetuating factor for sepsis. Dehydration and electrolytical changes can aggravate other organs hypoperfusion, predisposing the animal to organ failure.

Other organ systems like the central nervous system and the adrenal glands can also be affected by sepsis (Silverstein and Otto 2012; Osterbur et al. 2014).

1.3. Diagnostic approach

1.3.1. Physical exam

The clinical findings in animals suffering from sepsis reflect a systemic inflammatory state, with possible alteration of the vital parameters like temperature, heart rate and respiratory rate. These parameters should be measured upon admission and compared to see if they meet the SIRS criteria, as sepsis is diagnosed when the SIRS criteria are fulfilled and an infection is confirmed. The clinical signs tend to be unspecific as they correlate, not only to the organ system originally affected by the infectious agent, as well as any secondary organ dysfunctions mentioned above. Vomit, diarrhoea, dehydration, icterus and lethargy are just some of the common clinical signs observed.

Some dogs may already be in septic shock upon hospital admission. These will present either in the initial phase of shock, with pale mucous membranes, prolonged capillary refill time (CRT), and weak pulses, or in the hyperdynamic phase of shock. In this later stage, vasodilation subsists with resulting hyperemic mucous membranes, a decreased CRT (<1 sec), and strong or bounding pulses. Blood pressure should always bee part of physical examination of a suspected SIRS as hypotension may be present. (Hauptman et al. 1997; Drobatz et al. 2019).

1.3.2. Clinical laboratory findings

The diagnostic approach to a septic animal should include a complete blood count, biochemistry profile and coagulation tests. The hemogram may reveal abnormalities in different cellular lineages. Complete blood count most frequently reveal anaemia secondary to blood loss, haemolysis, oxidative damage and reduced erythrocyte production. Polycythaemia can also be present in hypovolemic animals due to haemoconcentration and splenic contraction. The majority of animals present with leucocytosis and band neutrophils and the blood smear reveal toxic changes to the neutrophils. Due to the immunosuppression and lymphocyte apoptosis it is also possible for lymphopenia and leukopenia to occur. Platelet consumption and DIC mean thrombocytopenia is also a usual finding (Girardot et al. 2016; Drobatz et al. 2019).

Biochemical abnormalities vary and reflect the organ dysfunctions taking place, either primarily affected by the infection or secondary to the inflammatory state. Common findings include hypoalbuminemia, glycemia alterations, hypocalcemia and hyperbilirubinemia. Hypoalbuminemia is a result of combined decreased hepatic synthesis and protein loss trough the gastrointestinal and urinary tract. Glycemia changes over the course of SIRS, with hyperglycaemia being more common due to cortisol and catecholamines release in response to stress and hepatic insulin resistance.

Hypoglycaemia ensues from increased consumption and decreased hepatic synthesis and reflects a more terminal stage of sepsis, in both dogs and cats. Haemolysis and cholestasis are responsible for the hyperbilirubinemia some animals present. Animals can develop azotaemia if chronic or acute kidney injury is present. In these patients urinalysis and urine culture should be carried out to assess any urinary tract infections, as kidneys can be the primary organ affected by the infectious agent (Sykes 2014a; Drobatz et al. 2019).

Coagulation tests should also be a part of the septic animal´s screening. In the initial hypercoagulable phase of DIC coagulation tests may show increased D-dimers and decreased concentration of activated protein C and antithrombin. With the development of the hypocoagulable state changes might include prolongated prothrombin time and activated partial thromboplastin time (Silverstein and Otto 2012; Drobatz et al. 2019).

1.3.3. Diagnostic imaging

Radiographs and ultrasound can help determining the source of infection causing the SIRS but can also elucidate the overall organ function and any secondary alteration. Different projections of thoracic radiographs (lateral and ventral dorsal) should be taken, especially in animals showing tachypnea or dyspnea. Images might show pulmonary patterns consistent with pneumonia or ARDS. The intervertebral disc space should also be evaluated since discospondylitis is also a possible cause of sepsis.

Abdominal ultrasound is a valuable tool, especially in animals with biochemistry values compatible with abdominal organ dysfunction. Ultrasound images can reveal primary lesions, like abscesses or septic effusions, and secondary lesion, like renal injuries and gastrointestinal ulceration. Any abnormal free fluid should be sampled for cytology, culture and susceptibility as it can help determine the origin of the infection and redirect the antibiotherapy (Silverstein and Otto 2012; Sykes 2014a).

1.3.4. Microbiologic test

The definitive diagnosis of sepsis implies the fulfilment of the SIRS criteria and the confirmation of infection, which require pathogen isolation and identification. First approach diagnosis must include imaging guided tissue and fluid sampling as well as cystocentesis urine sampling. Clinical history, presenting signs and lesion distribution must always direct the sampling, as it helps interpret the validity of the results. Cytology, histopathology, and both aerobic and anaerobic culture and sensitivity test, should all be performed in order to identify the infectious agent and help in the treatment decision

making. Ideally the samples should be taken before antibiotherapy is started but this is not an exclusion criteria (Hauptman et al. 1997; Drobatz et al. 2019).

Blood samples can also be useful if bacteraemia is suspected. Blood should be withdrawn from two different sites with at least 30 minutes apart and the skin should be prepared in a surgical fashion to prevent contamination (Sykes 2014a; Drobatz et al. 2019).

Polymerase chain reaction (PCR) is also a useful tool to identify infectious agents, especially when used in conjugation with culture. PCR assays are particularly useful in the detection of fastidious growing organisms. Antibody quantification can also be used to determine an acute response to infection (Miller et al. 2011; Sykes 2014a).

1.4. Treatment

1.4.1 Initial stabilization

The initial approach to sepsis must consider the early goal-directed therapy and medical management should focus on resolving the hemodynamic changes in the first hours upon presentation, especially in the high-risk patients (Gyawali et al. 2019).

One of the first steps toward the stabilization of a septic patient is to ensure tissues perfusion and oxygen delivery, preventing the development of MODS. Hypovolemic and distributive shock are the two most common phenomena contributing for the hemodynamic changes verified during SIRS. Therefore, intravenous fluid therapy should be initiated as soon as possible with the administration of crystalloids boluses, of up to 90mL/kg for dogs and 60mL/kg for cats. Isotonic crystalloids or hypertonic crystalloid solutions can both be used for initial resuscitation and it should be accompanied by clinical monitorization of heart and respiratory rate, blood pressure and urinary output, so the minimal amount of fluids needed to maintain adequate blood pressure is administered, thus avoiding overloading the animal. The use of colloids should be avoided as they have been proven to augment mortality in critical ill patients. Colloidal administration is associated with acute kidney injury (AKI) and coagulation dysregulation (Drobatz et al. 2019). Other transfusion therapies may be required depending on the clinical presentation for example severely anaemic animals may need packed red blood cells or fresh all blood transfusion. (Silverstein and Otto 2012; Dellinger et al. 2013; Drobatz et al. 2019).

1.4.2. Cardiovascular support

When animals present with septic shock, hypotension may be unresponsive to intravascular volume expansion alone and there is the need to resort to vasopressor

drugs to control the vasodilation. Vasodilation may present clinically as mucosal hyperaemia, decreased CRT and bounding pulses (Silverstein and Otto 2012).

Vasoconstrictor drugs usually used include vasopressin, dopamine, norepinephrine and epinephrine. Epinephrine has been associated with increased mortality when compared to norepinephrine and vasopressin and should, therefore, be avoided if other options are available (Minneci et al. 2004). Vasoconstriction might worsen the organ dysfunction by cutting the blood supply to some organ systems, vasopressin low-dose therapy is the safest drug choice as it spares cerebral, renal, pulmonary and mesenteric vessels constriction (Silverstein and Otto 2012; Drobatz et al. 2019).

Positive inotropic drugs, like dobutamine or pimobendan, may also be administered to help ensure tissue perfusion reduced by myocardial depression (Drobatz et al. 2019). Considering that critical illness-related corticosteroid insufficiency (CIRCI) causes vascular hyporeactivity, glucocorticoids may also be indicated in some cases of sepsis.

1.4.3 Antimicrobial therapy

Upon recognition of sepsis, antimicrobial therapy should be initiated as soon as possible, with intravenous antibiotic administration within the first hour being associated with higher survival rates. When possible, antibiotics should be chosen regarding possible pathogens involved, infection site and antibiotic resistance (Dellinger et al. 2013).

Initial approach, before culture and susceptibility results, includes the utilisation of a broad-spectrum antibiotics that should cover as many quadrants as possible. This empiric antibiotic coverage should only be maintained for up to 5 days, after which the antibiotherapy should be narrowed and adjusted according to the lab results (Drobatz et al. 2019; Gyawali et al. 2019).

Examples of first line antibiotics used in sepsis include: ampicillin and enrofloxacin, ampicillin and amikacin, ampicillin and cefoxitin, clindamycin and enrofloxacin, amoxicillin clavulanic acid and enrofloxacin. These options ensure coverage against Gram-positive and Gram-negative aerobes and anaerobes (Silverstein and Otto 2012).

Another step that helps with infection control is source control. This includes any procedure that physically contributes to infection control, either by removing the infectious site or by reducing the propagation of infection to adjacent tissues. Procedures involved in this step include surgical removal of infection focus and drainage of septic fluids (Gyawali et al. 2019).

1.4.4. Supporting care

Additional therapeutic measures after patient stabilization, should be directed to the organ dysfunctions taking place and adding good supportive care.

One organ system that requires attention in septic animals is the gastrointestinal (GI) tract, as it may contribute to the further release of bacteria into the bloodstream, contributing to the worsening of the infectious status. Gastrointestinal bleeding and stasis should be addressed with antiemetics (as maropitant, ondasetron), prokinetics (as metoclopramide), antiacids and sulcralfate. Enteral nutrition may also be helpful in restoring health to the intestinal villus (Silverstein and Otto 2012; Drobatz et al. 2019).

1.5. Prognosis

Sepsis is a serious condition with mortality rates higher than 50%, worse if shock is present (Silverstein and Otto 2012). Organs dysfunctions contribute to the mortality of animals, with prognosis being worse with the increase of organ systems affected (Kenney et al. 2010). Timely diagnosis and early treatment are key factors to maximize sepsis survival.

2. Canine Parvovirus

2.1. Etiology and epidemiology

Canine parvovirus enteritis is one of the most common infectious diseases affecting dogs. Canine parvovirus (CPV), are small nonenveloped DNA viruses. Two types, type 1 and type 2 are recognized, with type 2 being the most virulent form that underwent a series of mutations evolving into new strains: type 2a (CPV-2a), type 2b (CPV-2b) and type 2c (CPV-2c) spreading around the globe (Sykes 2014b).

The extremely resistant nature of the virus allows it to stay infective in the environment for long periods of time. CPV is expelled in the faeces of infected dogs and spreads via oronasal exposure. Even though the infection may occur in animals from any breed, age or sex with different individual response to infection, most of the acutely affected animals are unvaccinated puppies between the age of 6 weeks and 6 month, and mortality rates are high. Protection against the virus is long lived after infection, with maternally derived antibodies (MDA) being an effective protection for the first weeks of a neonate`s life. The most susceptible period is between the loss of maternal defences and the completion of the vaccination protocol. MDA protection declines with time and susceptibility to infection arise (Pollock & Carmichael, 1982). Rottweilers, Doberman pinschers, Labrador retrievers, American Staffordshire terriers, German shepherds, and

Alaskan sled dogs appear to be the breeds more susceptible to infection (Glickman et al., 1985; Houston et al., 1996; Silverstein and Otto 2012).

Multiple factors contribute for the severity of the infection. Clinical signs differ depending on the virus strain, host immunity and the presence of concurrent infections such as other enteric viral and parasitic infections (Sykes 2014b).

2.2. Pathogenesis

The CPV requires a mitotically active cell population to replicate, which makes young dogs (under 12 weeks) the target population. After oronasal exposure to CPV-contaminated feces, follows a 7 to 14 days incubation period. The virus first replicate in the oropharynx lymphoid tissue, thymus and mesenteric lymph nodes but, within 1 to 5 days, viremia occurs and hematogenous spread to the intestinal crypts of the small intestine takes place. Virus replication causes destruction of the germinal epithelium impairing the normal cell regeneration and causing villi collapse, this results in haemorrhagic diarrhoea and malabsorption. Shedding of the virus in the faeces starts as soon as 3 days post infection and can last for up to 4 weeks (Goddard and Leisewitz 2010; Sykes 2014b; Drobatz et al. 2019).

Viral infection within the uterus may result in reabsorption or abortion. When the virus affects neonates up to 2 weeks old, it can also target the mitotically active myocardial cells, causing viral myocarditis with signs of congestive heart failure and sudden death (Sykes 2014b).

2.3. Bacterial translocation and sepsis

Multiple factors contribute for the development of sepsis in CPV infections. Cellular destruction, intestinal hypomotility, dysbiosis, gut inflammation and tissue necrosis all contribute to the disruption of the gastrointestinal mucosal barrier, allowing Gram-negative and anaerobic bacteria translocation from the intestinal lumen to the bloodstream and bacteraemia to develop (Greene & Decaro, 2012; Kalli, 2016; Krentz & Allen, 2017).

Along with the mucosal barrier disruption, impaired immunity develops increasing the susceptibility to secondary infections. Marked leukopenia (mostly neutropenia and lymphopenia) is often observed in CPV infected dogs, as the virus also targets the mitotically active precursors of leukocytes and lymphoid cells of the bone marrow and lymphoid tissue like the thymus. Increased tissue demand and sequestration of neutrophils in the damaged GI mucosa also contributes for the neutropenia. Neutropenia

and bacteria overload impair the elimination of luminal bacteria from the bloodstream that occurs in healthy animals (Goddard and Leisewitz 2010; Kalli 2016)

The passage of viable bacteria or their products to extraintestinal sites can potentiate the development of sepsis, which increases the mortality rate of CPV infected animals since it predisposes to coagulation alterations, thrombotic events and MODS. With SIRS progression and the release of inflammatory mediators, the gastrointestinal barrier is compromised again, contributing to the cycle of bacterial translocation (Krentz & Allen, 2017).

2.4. Diagnostic Approach

2.4.1. Physical examination

The clinical signs of CPV-2 infection are unspecific and corelate to enteritis. In the initial phase of infection the dogs may present anorexia, lethargy and elevated rectal temperature, that later develop into gastrointestinal manifestations, including vomiting and diarrhoea. The faeces characteristics vary and can range from mucoid to haemorrhagic, with large volumes and liquid consistence. Abdominal pain is also a common finding and may be related to the severe enteritis or secondarily intestinal intussusception (Kalli 2016; Drobatz et al. 2019).

In consequence of the profuse vomiting and diarrhoea, and the resulting fluid loss, dehydration and hypovolemia may ensue. In these cases animals will present with impaired perfusion signs, including altered mucous membrane colour, tachycardia, prolonged CRT, weak pulses and altered mentation. Dogs that develop sepsis associated to bacterial translocation may present with clinical signs of SIRS or septic shock, which indicates a worst prognosis (Goddard and Leisewitz 2010; Drobatz et al. 2019).

2.4.2. Clinical laboratory findings

Leukocyte count on admission is of predictive value for 24h survival, with nonsignificant leukopenia being associated with 100% survival rate (Goddard et al., 2008). Leukopenia is the typical haematological change found in CPV-2 infected dogs. Destruction of hematopoietic linage precursors in the bone barrow, depletion of lymphoid organs and massive intestinal recruitment, all contribute for the low leukocyte count. Leucocytosis may also be present in a smaller number of animals and may be due to a bone marrow hyperplasia, compatible with a later stage of infection (Sykes 2014b; Kalli 2016).

Other haematological chances may include blood loss anaemia and alteration of the platelet count, with both thrombocytopenia and thrombocytosis being a possible finding.

Serum biochemical tests might reveal some unspecific alterations often including hypoalbuminemia and hypoproteinaemia, secondary to malnutrition and profuse vomiting and diarrhoea. Hypoglycaemia or moderate hyperglycaemia can also be present. Possible electrolyte abnormalities include hypokalaemia, hypomagnesemia, hyponatremia and hypochloraemia. Pre-renal azotaemia may ensue as dehydration progresses, animals that develop sepsis may also show other enzymatic changes reflecting organ systems dysfunction (Sykes 2014b; Kalli 2016).

Acid-base tests more commonly reveal acidosis, but alkalosis may also be present depending on the severity of the vomiting and diarrhoea (Goddard and Leisewitz 2010).

2.4.3. Microbiological test

The faecal parvovirus antigen ELISA is a test performed on the faeces obtained via rectal swab. Specificity higher than 90% and a practical use, makes it the most widely used test for CPV-2 enteritis. The test disadvantage is the low sensitivity that can vary between 18% and 60%. False negatives are associated with intermittent viral shedding, antigen neutralization by circulating antibodies and dilution of antigen on the stools. False positive results are rare but can happen 4 to 10 days after vaccination with modified live CPV vaccine. A strong clinical suspicion allied with a negative ELISA, should be followed by a more sensitive faecal antigen detection test including electron microscopy, viral isolation, faecal hemagglutination, immunochromatography, and PCR (Goddard & Leisewitz, 2010; Sykes, 2014b; Zoetis 2017).

Serology can also help in the diagnosis of a dog with typical clinical signs, if it reveals recently produced antibodies (immunoglobulins M). Care should be taken interpreting serological results, as positive results may be due to vaccination, maternally derived antibodies, or previous subclinical contact with the virus (Goddard and Leisewitz 2010; Kalli 2016).

2.5 Treatment

2.5.1. Fluid therapy

Early supportive care implementation is associated with higher survival rates. The first goal of the therapy is to correct dehydration and improve hemodynamic, as well as acid-base and electrolyte abnormalities correction. Intravenous (IV) fluid therapy is the

most effective way to restore the fluids lost through vomiting and diarrhoea, as subcutaneous fluid absorption is impaired in dehydrated animals. Intraosseous catheterization may also be considered. Aseptic measures should be taken when placing the catheter as the immune system is impaired in CPV-2 infected dogs (Goddard and Leisewitz 2010; Kalli 2016).

The initial fluid choice should be an isotonic crystalloid solution. Oncotic pressure and fluid deficits must be corrected according to the patient presentation and accompanied by monitorization of perfusion indicators including CRT, pulse and blood pressure. In puppies presenting hypovolemic, fluids rate must be enough to restore circulating volume in the first few hours upon admission. If shock is suspected 15-20ml/kg boluses can be given 15 minutes apart, until a shock dose of 80-90 ml/kg is reached. Once perfusion is restored IV fluid rate should be adjusted to cover maintenance requirements and fluid losses. Colloidal administration can be considered if perfusion restauration is refractory to crystalloids alone and if hypoproteinaemic peripheral edema occurs (Kalli 2016; Drobatz et al. 2019).

Since hypokalaemia and hypoglycaemia are commonly found in CPV-2 infected dogs, potassium chloride and 2,5%-5% dextrose supplementation can be required. Daily electrolyte and acid-base status monitoring is recommended, and abnormalities should be corrected accordingly. Fresh frozen plasma has also been indicated as part of the treatment protocol and, even though there is little prof of its efficacy, it is associated with higher survival rates (Goddard and Leisewitz 2010; Kalli 2016).

2.5.2 Antimicrobial therapy

Parvovirus enteritis predisposes for the development of sepsis and broad-spectrum antibiotic coverage should be warranted. The antimicrobial agents' choice must cover Gram-negative and anaerobic bacteria, as these are the most commonly found in parvovirus associated bacteraemia. Treatment protocols based on a penicillin with a beta-lactamase inhibitor, like amoxicillin clavulanate, or second-generation cephalosporin, can be effective single-agent therapies. Usually additional antibiotic coverage is warranted by the addition of aminoglycosides or metronidazole. Even though quinolones ensure a good anaerobic coverage, they should be used with precaution in growing dogs, in order to avoid cartilaginous damage (Goddard and Leisewitz 2010; Sykes 2014b; Kalli 2016).

2.5.3 Antiemetic treatment

Vomiting control helps preventing further fluid losses, increases patient comfort and allows oral nutrition and medication administration. Among the antiemetic options the more commonly used include metoclopramide, maropitant and ondansetron. Metoclopramide (a dopaminergic antagonist), used as boluses or as a constant rate infusion, is an efficient antiemetic drug but requires monitoring for increasing risk of intussusception. Serotonin antagonists like ondansetron, can be used as second line antiemetic treatment for uncontrollable emesis. Maropitant (an antagonist of neurokinin 1 receptors) is also a very effective antiemetic option, as it warrants both peripheral and central emetic pathways regulation. A multiple drug therapy might be necessary (Goddard and Leisewitz 2010; Kalli 2016).

2.5.4. Nutritional support

Being a GI disease, nil per os until clinical sings remission for 12 hours has been recommended in the past. Recent studies have shown that early administration of nutritional support via tube feeding is associated with faster clinical improvement, weight gain and gut barrier improvement, which could help prevent parvovirus associated sepsis. Small amounts of easily digestive food should be given early in the treatment of this animals (Goddard and Leisewitz 2010; Kalli 2016).

2.5.5. Pain management

Abdominal pain is usually present due to enteritis or secondary intussusception, pain relief should be administered as pain negatively impacts the appetite and delays recovery. Commonly used drugs include buprenorphine (a partial agonist of μ-opioid receptors) and butorphanol (an antagonist of K-opioid receptors). Maropitant, apart from the antiemetic effect may also have a pain relief effect, since is also a P substance blocker, which is involved in visceral nociception. Non-steroidal anti-inflammatory drugs should be avoided, as they can further the GI mucosal damage via COX-1 inhibition (Kalli 2016).

2.6. Prognosis

Survival rates of dogs with CPV-2 enteritis vary depending on individual characteristics and immune competence, and the treatment offered. Considering this, survival rates can range from 9% to 90%. (Sykes 2014b).

Factors that have been associated with higher mortality include initial leukopenia, lymphopenia and neutropenia, and meeting SIRS criteria. Hospitalization time also

varies, with vomiting, lethargy, lymphopenia and hypoalbuminemia all being associated with prolonged hospitalization (Kalli et al. 2010). Survival has been associated with early positive shifts in leukocyte count. Being mostly an acute process, animals that survive the first 3 to 4 days of treatment tend to make full recoveries.

Prognosis is worse if complications arise. Complications include bacteraemia and sepsis, aspiration pneumonia, oesophageal stenosis and intussusception (Sykes 2014b).

2.7. Prevention

Proper immunization is the most effective method of CPV-2 infection prevention. Initial passive immunity is warranted by maternally derived antibodies, most of which are obtained from the colostrum. It is also important to note that MDA interfere with proper immunization and should be considered when planning the vaccination of a puppy. Maternal CPV antibodies have a half-life of approximately 10 days and will have waned enough to allow effective vaccine protection after 8 to 12 weeks (Goddard and Leisewitz 2010; Kalli 2016).

The most recent vaccination guidelines classify the type 2 parvovirus vaccine as a core vaccine, meaning all dogs, regardless of the circumstances, should receive it. Recommendation is to start the vaccination protocol with a modified live vaccine at 6 to 8 weeks of age and then every 2 to 4 weeks until, at least, 16 weeks of age. If the initial dose is due after 16 weeks of age, two doses of the vaccine should be administered 2 to 4 weeks apart, even though one dose is considered protective. Revaccination should be given at 6 months or 1 year of age, and then once every 3 years (Day et al., 2016). Post infection immunity is effective and long lived.

Despite the efficacy of the vaccination, other health care measures should be taken to prevent infection, including disinfection of faeces exposed surfaces and objects, quarantine of newly introduced animals, isolation of infected dogs and reduction of stress factors (Goddard & Leisewitz, 2010; Kalli, 2016).

3. PIRO: staging of sepsis

3.1. PIRO (Predisposition, Insult, Response, Organ dysfunction/failure)

Even with the application of consensual treatment guidelines, sepsis is still one of the leading causes of death, with some patients not responding to the proposed treatment. The heterogeneity of the infective process and of the individual immune

response to it, makes prognosis prediction and treatment choice difficult in these animals (Rathour et al. 2015).

In order to try and stage septic patients by both their risk of mortality/adverse outcome and their potential to respond to treatment, in 2001 (the international sepsis definitions conference) a new stratification system was introduced, the PIRO. This system allows to stratify patients based on their predisposing conditions, the nature and characteristics of the insult, the extent of the host immune response to it, and the associated organ dysfunction (Levy et al. 2003). The purpose of this system is to help in the enrolment of individual in clinical studies and prognosis of septic patients, allowing to adapt the therapy offered and improve survival.

3.2 Predisposition

When considering predisposition, all factors present previous to the onset of the acute illness and that might influence its incidence or outcome, should be considered. This include genetic predisposition, acquired comorbidities and concomitant diseases (Levy et al. 2003; Rathour et al. 2015). Apart from genetic factors, that include sex and breed predisposition, advanced age and concomitant disease including liver disease, neoplasia, heart conditions and chronic renal failure were all associated with higher mortality and should hence be considered as predisposing factor for sepsis prognosis (Rubulotta et al. 2009; Howell et al. 2011; Granja et al. 2013).

3.3. Insult / Infection

When considering septic animals, the insult is the infection that triggers the SIRS. Infection characteristics like extent, location and associated pathogen, can all have an impact on the outcome. Human medicine studies have linked higher mortality to sepsis originated from pulmonary, gastrointestinal and central nervous system infections, when compared to soft tissue or skin infections. The virulence of the infecting organism can also impact the outcome, with more virulent agents being linked to higher mortality. (Levy et al. 2003; Cohen et al. 2004; Opal 2005)

3.4. Response

The host response to the infectious insult is what defines sepsis. Since it´s SIRS that triggers the organ systems dysfunction found in septic patients, mortality is closely related to the individual response to an infection. Many of the factors mentioned above, including genetic factors and concomitant disease, can influence the host immune status and therefore the immune response mounted (Opal 2005).

Physiological parameters used to evaluate the extent of the septic patient´s response to infection (the SIRS criteria) include temperature, heart rate, respiratory rate and leukocyte count, as well as circulating biomarkers levels, can help determine the extent of the inflammatory response. These have prognostic value, as an increased magnitude of the inflammatory response has been correlated with higher mortality. (Opal 2005; Granja et al. 2013).

Individual response evaluation allows for better treatment choice, as hyper responsive and hypo responsive individuals should be offered different therapy options (Gerlach et al. 2003).

3.5. Organ dysfunction

The host inflammatory response ultimately results in organ dysfunction, with patients developing multiple organ failure being more likely to die than those who develop less or none. When exposed to the same infectious stimuli, different septic hosts can develop different organ systems dysfunction. It is also important to distinguish primary organ dysfunction, caused by direct infectious insult, and secondary organ dysfunction distant from the infection site, caused by the inflammatory response. Premorbid conditions may affect the way the disease progresses, predisposing to organ failure (Levy et al. 2003; Opal 2005). Any infection should be addressed as a possible sepsis, even with no signs of organic dysfunction, as it might be present but occult (Singer et al. 2016).

Organ failure scoring systems can be integrated into the PIRO evaluation and help assess the magnitude of the organ dysfunction present. The total sequential organ failure assessment (SOFA) score has been proven to be related to mortality (Granja et al. 2013). Other method for evaluating organ dysfunction is assigning scores if the organic parameters go above a certain predetermined level (Howell et al. 2011).

4. Other outcome prediction systems

4.1. APACHE (Acute Physiology and Chronic Health Evaluation)

The APACHE takes into account physiological parameters and preadmission conditions, in order to classify acutely ill patients accordingly to the severity of the condition and probability of mortality. A score is given depending on the alteration of physiological parameters that reflect the function of the major physiological systems (neurological, cardiovascular, haematological, renal, respiratory and gastrointestinal and metabolic), as well as the patient recent medical record (Knaus et al., 1981). APACHE II

and APACHE III where later adapted from the original to improve the system outcome prediction ability.

The main disadvantages associated to APACHE are lack of a specific design for septic patients and the need for previous medical records that may not readily obtainable (Macdonald et al. 2014).

4.2. SOFA (Sequential/ Sepsis-related Organ Failure Assessment)

Organ failure is a major cause of mortality in septic patients. In 1994 the SOFA was created as an attempt to objectively quantify the degree of organ dysfunction. Scores are given upon changes on physiological parameters that translate organ systems dysfunctions, the higher the score the more severe the dysfunction is and the poorer the outcome. Patients with a SOFA score higher than 2 are more likely to die (2-25 times) than those with a SOFA score lower than 2 (Singer et al. 2016). Apart from helping to understand the progress of organ failure during sepsis, the SOFA classification system allows to evaluate the effects of the treatment applied, as well as helping with the characterization of the patients upon presentation (Moreno et al. 1996; Innocenti et al. 2018).

Not being designed with the intent to predict mortality, but rather as a tool to describe acute illness complications, it can used as a complement to other staging systems. The quick SOFA (qSOFA) was introduced later to allow a quicker, bedside evaluation of critically ill patients, without the need to wait for laboratory results.(Singer et al. 2016)

4.3. MEDS (Mortality in Emergency Department Sepsis)

Septic patients present to the emergency department should be assessed for severity and mortality prediction without the need to look for information not readily available, which allows for prompt clinical decisions and treatment changes (Shapiro et al. 2003).

When applied to septic patients, MEDS have been proven not only to have a poor accuracy for predicting mortality but also to underestimate mortality risk when early goal directed therapy is applied (Jones et al. 2008). The fact that the virulence of the pathogen is not taken into account, limits the accuracy of this staging system (Macdonald et al. 2014).

III – Canine Parvovirus and Sepsis: SIRS criteria evaluation and implementation of a PIRO classification

1. Objectives

The main objective of the current study was to assess the prognostic value of the presenting vital signs as well as evaluate the possibility of stratifying and classify septic animals accordingly to a proposed PIRO classification system, using Parvovirus infection as a natural model for sepsis study. This could help assess prognosis and take part in the clinical decision making, as well as helping in the enrolment of study populations in future sepsis related studies.

2. Materials and methods

2.1. Inclusion criteria

The animals enrolled in this study included all canine patients hospitalized in the IDIU of the HEV-FMV-UL from November 2013 until June 2019, with a positive diagnosis of parvovirus enteritis (considered positive after ELISA or PCR fecal antigen detection), with complete blood count and biochemistry results available, clinical exam registered and known outcome (discharge or death). The dogs were subjected to two sets of SIRS criteria and to a proposed PIRO classification, upon admission. Both SIRS criteria, all individual variables of the PIRO and the total PIRO score where then correlated with the outcome.

All animals that did not fulfil the inclusion criteria or had concomitant diseases capable of causing gastrointestinal signs were excluded.

2.2. SIRS classification and PIRO score

2.2.1. Data recovery

All information required about the animals' clinical history was collected from both veterinary hospital's management software, QVet® and GuruVet®, and some information was retrieved from the isolation unit archived paper sheets. Some of the information used for the present study was already gathered in a previous work by Prata (2017).

Both clinical monitoring data and analytical parameters were included in the study. The clinical parameters included heart rate, respiratory rate, temperature, CRT and mucous membrane colour. The analytical parameters included data collected from the hemogram including leucocyte count, platelet count and information retrieved from biochemistry included albumin, creatinine, urea, bilirubin, ALP and ALT.

2.2.2. Classification criteria

In order to evaluate if a systemic inflammatory response syndrome was taking place, the animals were subjected to two sets of criteria. The first set, described above in table 1, was denominated SIRS 1991, and considered SIRS criteria as they were originally proposed and as they are currently used in clinical practice. The same evaluation using the same criteria was repeated, but SIRS was only considered upon CRT or mucous membrane colour alteration. This subset of criteria was denominated SIRS 2001 and was created in an attempt to increase the specificity of said criteria, as suggested by the paper published in the sequence of the SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference in 2001 (Levy et al. 2003).

The proposed criteria applied for the PIRO scoring, and for each of its' individual components - P, R and O - were extrapolated from an array of bibliographic founts, from both human and veterinary medicine. Said criteria were already compiled in a previous study (Prata. 2017). The independent influence of each of the considered classification parameters on the outcome was not evaluated.

The parameters proposed for each of the PIRO´s components are described below in tables 2, 3 and 4. For predisposition (P) age, breed and vaccination status were considered. Response (R) was considered to be characterized by temperature, heart rate, respiratory rate and leucocytes count. As far as organ dysfunction (O) is concerned, biochemical and clinical markers of renal, cardiovascular, respiratory, hepatic and coagulation system dysfunction were included. Since only parvovirus infected dogs were enrolled in this study, all animals had the same infection (I) score (equal to 1). The total PIRO score was obtained by adding all of the PIRO´s components.

Table 2. Proposed predisposition (P) scoring criteria for Parvovirus infected dogs, considering age, breed and vaccination status. (Glickman et al., 1985; Houston et al., 1996; Goddard & Leisewitz, 2010; Greene & Decaro, 2012; Prata, 2017).

	Parameters	Score
Age	<6 weeks	1
	>6 weeks ≤6 months	3
	>6 months	2
Breed	Toy Poodle and Cocker Spaniel	1
	Undefined	2
	Rottweiler, Labrador Retriever, American Staffordshire Terrier, German Shepherd, Alaskan Malamute	3
Vaccination Status	Complete Primary Vaccination	0
	Incomplete/Incorrect Primary Vaccination	2
	Not Vaccinated or Unknown Vaccination Status	3

Table 3. Proposed response (R) scoring criteria for Parvovirus infected dogs, considering temperature, heart rate, respiratory rate and leucocytes count. (Rijnberk & Stokhof, 2009; Boag, 2011; Sykes, 2014a; Marshall & Sweeney, 1990; Prata, 2017)

Criteria	Score			
	0	**1**	**2**	**3**
T (ºC)	37.8 - 39.4	39.5-40.4	36-37.7 or 40.5-41.4	<36 or >41.4
HR (bpm)	60-140	141-150	151-170	<60 or >171
RR (bpm)	10 - 30	31-40	41-50	>50 ou <10
Leucocytes Count (cells/µL)	6000 - 16.000	4.200 - 5.999 or 16.001-20.800	2.940 - 4.199 or 20.801 - 27.040	<2.939 or >27.041

Legend: T, temperature; ºC, degrees Celsius; HR, heart rate; bpm, beats per minute; RR, respiratory rate; bpm, breaths per minute; cells/µL, cells per microlitre

Table 4. Proposed organ dysfunction (O) scoring criteria, based on clinical and analytical parameters. Each evidence of organ system dysfunction equals to 1 point.

Dysfunction	Criteria
Renal	Creatinine>1.64 mg/dl; Creatinine>1.64 mg/dl and Urea>56 mg/dl
Cardiovascular	Hypotension requiring vasopressor drugs administration
Respiratory	Need for oxygen or ventilation supply, ARDS
Hepatic	Bile acid>25 µmol/L (post-prandial) and/or Bilirubin>0,41; ALT>130 U/L 37ºC e ALP>337 U/L 37ºC; Albumin < 2.1 g/dl
Coagulation alterations	Platelets≤100.000/µL

Legend: mg/dl, milligram per decilitre; g/dl, gram por decilitre; ARDS, Acute Respiratory Distress Syndrome; ALT, alanine aminotransferase; ALP, alkaline phosphatase; U/L 37ºC, units per litre at 37 degrees Celsius; µL, microlitre. The considered values correspond to the upper limit considered by the Professor M. Braço Forte clinical analysis laboratory (where all the blood samples were analysed).

2.3. Statistical analysis

All the collected data were recorded using Microsoft Office Excel 2016. The intended statistical tests were implemented using the computer program R, version 3.4.0. (R Core team. 2015).

2.3.1. SIRS

To evaluate the correlation between the fulfilment of the SIRS criteria and the outcome, all animals were classified according to the original SIRS 1991 and to the proposed SIRS 2001 criteria. Both results were separately subjected to the Fisher's exact test. For the statistical results analyses a 95% confidence interval was considered.

2.3.2. Total PIRO, Predisposition (P), Infection (I), Response (R) and Organ Dysfunction (O)

To evaluate the correlation between the PIRO scoring and the outcome, the scores for total PIRO and for each of its' components were divided into two groups, that were later correlated with the outcome. For each variable one of the groups contained the animals in the lower half of the values and the other comprised the animals in the upper half.

The total PIRO score ranged from 7 to 19. The sample was divided into two groups, according to their total PIRO score. The groups formed were "0-10" and "11-20". The "0-10" group was composed of 20 animals and the "11-20" group was composed of 52 animals. The scores for predisposition (P) ranged between 4 and 9. Two classes were created, one "0-5" and the other "6-10", the classes comprehended 7 and 65 dogs, respectively. Infection (I) score was 1 for all the animals enrolled, as Parvovirus infection was considered to be the only sepsis inducing factor in this sample. The response (R) scores ranged from 0 to 11. The classes created were "0-6", with 59 dogs, and "7-12", with 13 dogs. Finally, concerning the organ dysfunction (O) the sample included 59 dogs with a score of "0" and 13 animals with the score of "1", so those were the two classes considered.

The Fisher's exact test was applied to access the correlation between the scores and the outcome. A 95% confidence interval was considered.

3. Results

3.1. Sample characterization

The sample included data collected from 72 dogs hospitalized in the IDIU of the HEV-FMV-UL with confirmed Parvovirus infection. Concerning the outcome, 59 (81.9%) dogs were discharged and 13 (18.1%) dogs died. Regarding gender, 42 (58.3%) were male and 30 (41.7%) female. The majority of the dogs, 52 (72.2%), fell under the described susceptible age group of over 6 weeks and under 6 months, 12 (16.7%) dogs were over 6 months old, 5 (6.9%) were under 6 weeks old and 3 (4.2%) were of unknown age. As far as vaccination status is concerned, most dogs, 37 (51.3%), had no vaccination history, 29 (40.3%) had an incomplete vaccination record, 4 (5.6%) had an unknown vaccination history and only 2 (2.8%) were considered to have a complete vaccination status for Parvovirus infection. This study population breed distribution is as described in table 5.

Table 5: Sample characterization of the animals subjected to SIRS criteria and to the PIRO classification.

Gender	**n (%)**	**Age**	**n (%)**	**Vaccination Status**	**n (%)**
M	42 (58.3)	>6 weeks ≤6 months	52 (72.2)	Not Vaccinated	37 (51.3)
F	30 (41.7)	>6 months	12 (16.7)	Incomplete Vaccination	29 (40.3)
		<6 weeks	5 (6.9)	Complete Vaccination	2 (2.8)
		Unknown	3 (4.2)	Unknown	4 (5.6)

Breed	**n (%)**
Undefined	31 (43.1)
Labrador	10 (13.9)
Yorkshire Terrier	5 (6.9)
French Bulldog	4 (5.6)
Beagle	3 (4.2)
German Shepherd	2 (2.8)
Shiba Inu	2 (2.8)
Alaskan Malamute	1 (1.4)
Azores Catle Dog	1 (1.4)
Bull Terrier	1 (1.4)
Cocker	1 (1.4)
Dachshund	1 (1.4)
Golden Retriever	1 (1.4)
Great Dane	1 (1.4)
Rafeiro do Alentejo	1 (1.4)
Rhodesian Ridgeback	1 (1.4)
Rottweiler	1 (1.4)
Spitz	1 (1.4)
Poodle	1 (1.4)
English Setter	1 (1.4)
Boxer	1 (1.4)

Legend: n, number of animals; %, percentage.

3.2. SIRS criteria

The results from the Fisher's exact test, applied to both SIRS criteria, can be consulted in table 6. Considering that the p value for SIRS 1991 was 0.352, no significant statistical association was found between the fulfilment of the SIRS 1991 criteria and the

outcome. However, when considering the new proposed SIRS criteria (SIRS 2001), requiring CRP or mucous membrane colour alteration, a p value below the significance level considered of 0.05 was found, implying a significant statistical association was found between the SIRS 2001 criteria and the outcome (OR = 4.09, p = 0.0242). An odds ratio of 4.09 suggests that dogs fulfilling the SIRS 2001 criteria upon admission are approximately 4 times more likely to die than those who do not. (The Fisher's exact test tables for both SIRS criteria set can be consulted in the appendices 5 and 6.).

Table 6. Fisher's exact test results for the evaluation of the correlation between the different SIRS criteria and the outcome. A 95% confidence interval was considered

	p value	Odds Ratio	95% confidence interval
SIRS 1991	0.352	2.21	0.507 - 13.735
SIRS 2001	0.0242	4.09	1.044 - 17.109

3.3. PIRO score

The results from the Fisher's exact test performed to evaluate the correlation between the different PIRO variables and the outcome are presented in Table 7. (The Fisher's exact test tables for all the PIRO´s elements can be consulted in the appendices 1 to 4.).

The p value obtained for Predisposition (P) was 1, for Response (R) and Organ dysfunction (O) was 0.1135 and for the total PIRO was 0.093. Considering all the p values obtained were higher than the considered significance level of 0.05 no significant statistical association was found between any of the PIRO´s elements, neither the total PIRO score, and outcome. Additionally, to explore the possibility of using the SIRS criteria as fast decision-making tool, a Fast-and-Frugal tree (FFT) was created using the criteria considered to characterize the Response (R) element. This FFT, which is shown in Figure 1, revealed a sensitivity of 92% and a specificity of 29%.

Table 7. Fisher's exact test results for the evaluation of the correlation between the different PIRO variables and the outcome. A 95% confidence interval was considered.

	p value	Odds Ratio	95% confidence interval
Predisposition (P)	1	0.67	0.013 - 6.329
Response (R)	0.1135	0.294	0.065 - 1.409
Organ Dysfunction (O)	0.1135	0.294	0.065 - 1.409
Total PIRO	0.093	0.161	0.004 - 1.226

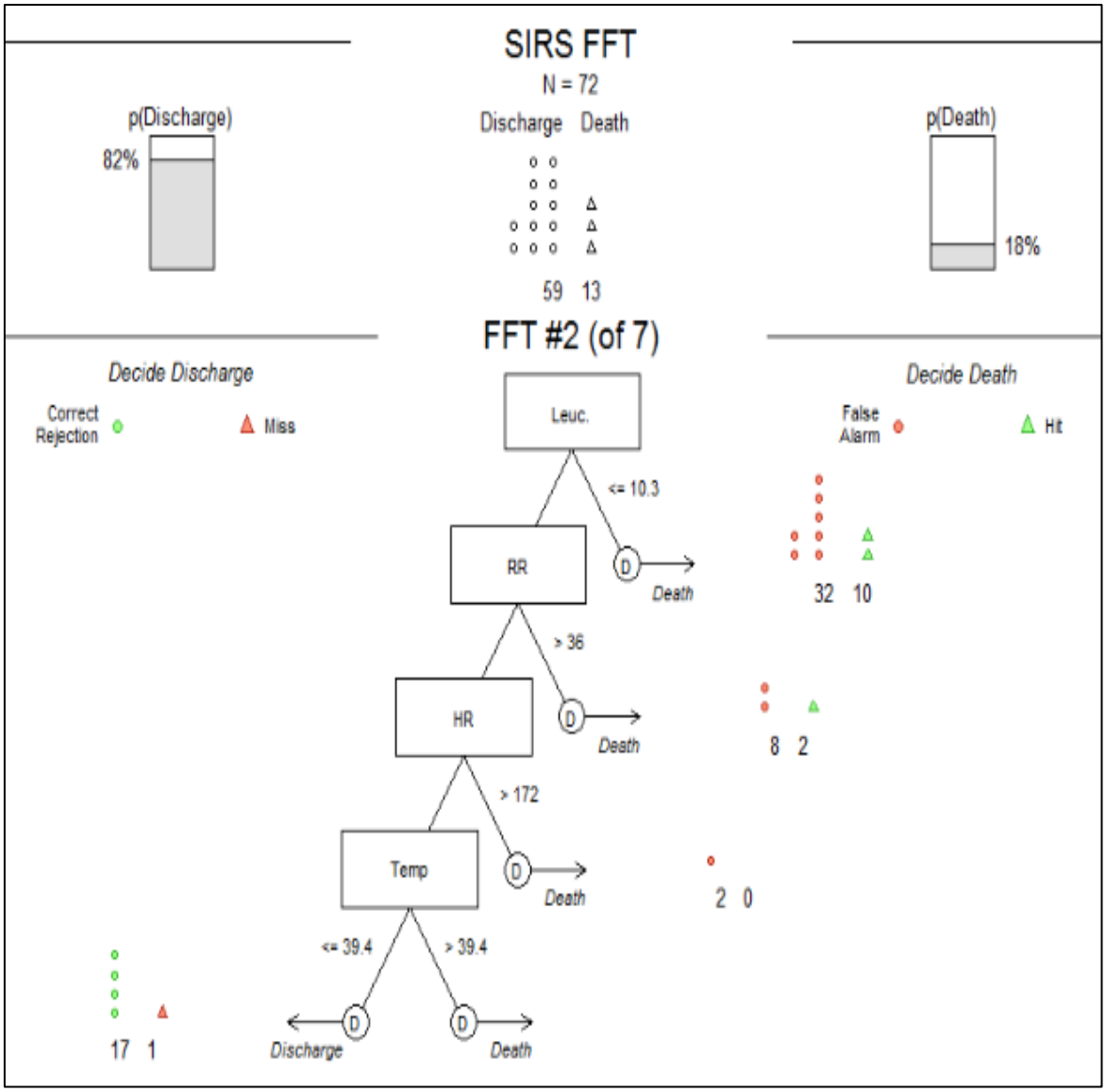

Figure 1: Example of Fast-and-frugal Tree for the Response (R) element of PIRO.

Legend: RR, respiratory rate; HR, heart rate; Leuc., leucocytes; Temp., temperature; Correct rejection, correct discharge prediction; Miss, incorrect discharge prediction; False alarm, incorrect death prediction; Hit, correct death prediction.

4. Discussion

4.1. SIRS

Since it was first introduced in 1991 for human medicine, and later adapted for veterinary medicine, the SIRS concept has been widely accepted and used by both clinicians and researchers to identify sepsis. On a 2006 sepsis survey, 80% of veterinarians acknowledged to use these criteria to diagnose sepsis (Otto 2007). One of the first studies to propose SIRS classification criteria for animals and assess their ability

to accurately diagnose sepsis was conducted by Hauptman et al. (1997). The meeting of at least two of the four proposed criteria as a diagnosis method revealed a 97% sensitivity and a 64% specificity. Even though the high sensitivity reported indicates that almost all septic animals are detected, the low specificity implies an over diagnosing of sepsis when using the proposed criteria (36% false positives). Despite the results obtained, the author still considered the proposed criteria as useful for sepsis diagnosis, suggesting that is better to over diagnose than to miss animals with less evident sepsis manifestations.

According to the most recent sepsis consensus, the current definition requiring 2 or more SIRS criteria to be met it is not suitable for sepsis diagnosis, with low sensitivity and specificity being reported in multiple studies (Singer et al. 2016). The same weaknesses can be assumed to be present when considering SIRS classification criteria for animals, with most clinicians resorting to the fulfilment of at least 3 of 4 criteria and not the recommended 2 of 4, in an attempt to improve the diagnosing accuracy (Otto 2007).

In this study, when the first set of criteria were applied (SIRS 1991), no significant statistical association was found between the fulfilment of the criteria and the outcome ($p = 0.352$). Multiple studies focusing on the outcome prediction ability of the SIRS criteria have been published with conflicting results. Mantione and Otto (2005), on a study population of CPV infected dogs, reported that SIRS at admission time did not consistently correlate to a negative outcome, only its´ persistence during hospitalization. Kalli et al. (2010) also took on a study population of CPV infected dogs and assessed which factors were associated with a negative outcome, reporting a higher mortality rate for dogs with SIRS at the time of admission ($p < 0.0001$), with puppies being 100 times more likely to die if presenting with SIRS. Okano et al. (2002) also reported SIRS to be a negative prognosis indicator ($p < 0.01$). The discrepancy between results may reflect the lack of a universal and validated classification criteria to characterize dogs´ systemic inflammation, with different studies reporting distinct results upon considering different classification criteria.

Systemic inflammatory response syndrome criteria are pointed has not being specific enough to be useful in sepsis diagnosis. SIRS can be present in response to an infectious stimulus but other traumatic or inflammatory conditions (including pancreatitis or extensive burns) can also result in the same clinical presentation. The poor discriminant validity of the SIRS criteria implies their presence in many hospitalized patients without necessarily implying a negative outcome. In an attempt to enhance the specificity of said criteria on human sepsis diagnosis, the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference Task Force

proposed a list of possible signs of inflammatory response to infection that could be added to the existing SIRS criteria hence augmenting their specificity (Levy et al. 2003). In the present study one of the proposed variables was added to the existing SIRS criteria in order to create a new set of criteria denominated SIRS 2001, according to which the SIRS criteria would only be applied upon an altered capillary refill time or a mucous membrane colour alteration.

When the new classification criteria were applied to the sample population a significant statistical association with the outcome was found (OR = 4.09, $p < 0.05$). According to our results, dogs that met the SIRS 2001 criteria on admission were about 4 times more likely to die than those who didn't. The improved predictive value verified may be due to an increased specificity of the criteria applied, as they might be indicators of hemodynamic instability and tissue perfusion compromise. Even though none of the proposed parameters is specific for sepsis they can be indicators of an onset of organ dysfunction, which goes into agreement with the most recent sepsis definition as a life-threatening organ dysfunction caused by a dysregulated host response to infection, thus helping to increase the diagnosing criteria specificity (Singer et al. 2016). It is important to note that the modifications made could imply a decreased sensitivity, considering that mucous membrane alterations can be absent in the presence of sepsis if the hemodynamic function is not severely affected, potentially leading to false negatives (since no moderate hypovolemia is detected).

The low specificity of the variables chosen to characterize the inflammatory response on this population can be responsible for the results concerning the 1991 criteria. The same limitations described below for the response (R) element can be pointed (higher respiratory and heart rate in puppies and lower leukocyte count due to viral destruction (Fransson et al. 2007; Savigny and Macintire 2010)), as the same criteria were used to characterize both. This could have accounted for an incorrect fulfilment of the SIRS criteria, thus resulting in a non-significant p value.

The real sensitivity and specificity of both SIRS criteria considered cannot be assessed because no gold standard diagnostic test exists. This limitation is found not only in this study but throughout sepsis investigation.

4.2. Predisposition (P)

Predisposition takes into account all the factors that are present before the onset of sepsis and that may influence the outcome upon an infectious insult. Human medicine studies have shown genetic differences in sepsis related mortality depending on gender and race (Martin et al. 2003; Sakr et al. 2013). Apart from genetic factors, both age and medical co-morbidities have also been reported to be related to in hospital mortality in

various studies. Factors associated with increased sepsis related mortality in humans include: advanced age (over 60/65 years old), chronic obstructive pulmonary disease, chronic renal failure, chronic haematological disease, neoplasia with or without metastases and chemotherapy (Howell et al. 2011; Granja et al. 2013).

Further veterinary medicine directed studies are still needed to comprehend the influence of predisposing factors in the systemic inflammatory response of animal species. One study concluded that geriatric dogs had a weaker IL-10 production upon bacterial LPS stimulation, which may translate into an exacerbated inflammatory response and greater risk of mortality when compared to younger dogs (Deitschel et al. 2010). Breed has also been shown to influence the inflammatory response, with one study concluding that Parvovirus susceptible breeds (Rottweilers and Doberman pinschers) had an elevated TNF-α production in response to LPS stimulation when compared to mixed breeds (Nemzek et al. 2007).

For the current study the predisposing factors included were age, breed and vaccination status. The sample was composed mainly by undefined breed dogs (43%) and 14 dogs were considered to have a breed predisposition for Parvovirus enteritis (10 Labrador, 2 German Shepherd, 1 Rottweiler and 1 Alaskan Malamute). Most dogs (72%) were aged between 6 weeks and 6 months and the majority of the animals included had no vaccination history (51%) or an incomplete/incorrect vaccination (40%). As far as age and vaccination status are concerned, this sample reflects what has been described in literature about Parvovirus infection predisposition, with young not vaccinated dogs being the most susceptible to infection.

In the present study no significant statistical association was found between predisposition and outcome ($p = 1$). This result differs from most human medicine studies that have shown a significant statistical association between predisposition and outcome, with Granja et al. (2013) and Howell et al. (2011) both reporting a strong correlation with mortality ($p < 0.001$). When considering the predisposition (P) discriminatory ability for predicting outcome, a poor to fair accuracy have been described with Rathour et al. (2015) reporting an area under the receiver operating characteristics curve (AUC-ROC) of 0.79 and Granja et al. (2013) reporting an AUC-ROC of just 0.66. These results may indicate that predisposition alone is not a good outcome prediction element but, since it is indeed linked to the outcome, it should be included in the total PIRO classification in order to improve its overall accuracy.

Population characteristics may be accountable for the discrepancy of results obtained in this study and the ones previously cited. Factors including the overrepresentation of animals within the susceptible age group or the underrepresentation of death among the susceptible breeds may all have contributed,

with only 2 animals considered to be breed predisposed (2 Labradors) dying. It might also be the case that the parameters chosen to characterize the dogs´ predisposition are not the most adequate to evaluate the predisposition influence on sepsis mortality.

On the other hand, these results come into agreement with the results observed in a more recent study on Parvovirus affected dogs, where no correlation between any particular breed or age group and the outcome was found. Only reporting that purebreds are 2.5 times more likely to develop the disease (Kalli et al. 2010).

4.3. Infection (I)

For the current study Parvovirus was considered to be the only infection inducing element and, since all the animals were considered to have the same infection score of 1, its correlation with the outcome was not statistically evaluated.

Human medicine studies have shown a strong correlation between the severity of infection and the outcome (Howell et al. 2011; Granja et al. 2013). Factors like the type of microorganism, the infection focus, and antibiotic therapy applied can all influence the patient's systemic response and ultimately the outcome. Different classifications have been proposed for the infection score. One example of a possible classification system for the infection element of PIRO is the one employed by Rathour et al. (2015), where the types of microorganism as well as the infection site were considered, with different scores being attributed for respiratory tracts infection, urinary tract infection and central nervous system infections. Mortality has been shown to be positively related to: fungal infections, positive blood cultures, antibiotic therapy implementation and health care associated sepsis (Granja et al. 2013).

Further studies are needed in the veterinary medicine field to better characterize how the infection affects outcome. Though it is known that the clinical signs of septic animals can vary depending on the location and extent of the infection, a case report concerning a dog with a history of brain abscess and bacterial endocarditis secondary to a Staphylococcus sp. infection that only met 2 of 4 SIRS criteria, disclosed that the systemic signs of inflammation may not directly correlate with the infection severity (Bach et al. 2007; Otto 2007).

4.4. Response (R)

The individual inflammatory response to an infectious stimulus is inherent to the sepsis development, as it is the host dysregulated response that triggers the organ dysfunction observed. The inflammatory response is also accountable for the clinical changes observed during sepsis and can be of predictive value. Variables proven to be

related with mortality include heart and respiratory rate, leucocyte and band neutrophils count (Howell et al. 2011; Granja et al. 2013; Rathour et al. 2015).

For this study the factors considered for the characterization of the host inflammatory response were the SIRS diagnosis criteria (HR, RR, T and leucocytes count). No significant statistical association was found between response (R) and outcome ($p = 0.1135$). These results differ from what have been reported in human medicine studies, in which response was positively associated with mortality. Granja et al. (2013) and Howell et al. (2011) both observed a strong correlation between response and outcome, with a $p = 0.002$ and a $p < 0.001$ being reported respectively. On another study the only two response variables related with hospital mortality were increased respiratory rate (>20 breaths/min) and bandemia (>5% immature band neutrophils). In said study a fair outcome prediction accuracy was reported for the response element, with an AUC-ROC = 0.74 (Rathour et al. 2015).

The observed discordance between results may be due to the low specificity of the variables chosen, as they can be altered by non-pathological factors such as pain or anxiety. The specificity may even be lower when considering the population in question, since Parvovirus predominantly affects puppies which usually exhibit a higher respiratory and heart rate when compared to older dogs (Fransson et al. 2007; Kalli et al. 2010). Also, for the leukocyte count the specificity may be diminished when considering a Parvovirus infected population. A lower leukocyte count may only reflect the virus effect on the white blood cell population, with bone marrow and lymphoid tissue precursor cells being targeted and destroyed, and not being necessarily SIRS related (Savigny and Macintire 2010).

In an attempt to improve the outcome prediction ability of the response element, one should consider incorporating biochemical markers of inflammation such as inflammatory cytokines (IL-6 and TNF), CRP or coagulation proteins (Howell et al. 2011; Rathour et al. 2015). The retrospective nature of the present study made it impossible to include biochemical markers of inflammation, as they are not part of routine biochemical analyses.

One interesting use of the SIRS criteria in sepsis outcome prediction, thus helping in a faster decision making for both clinicians and researchers, would be the construction of a Fast-and-Frugal Tree (FFT). Figure 1 represents one of the possible FFT that could to be used to help in the characterization and scoring of the response (R) element of the PIRO system. It is important to emphasize that this tree was not tested on another sample as it is recommended, and so the results may only reflect the tree´s performance for this particular sample (Phillips. 2017). This FFT has a sensitivity of 92% and a specificity of 29%. In our study sensitivity was primed over specificity in the making of

this FFT, as it is in our opinion more important to have a higher sensitivity when considering life threatening conditions like sepsis, in order to diminish the number of false negatives and the risk of missing severely ill animals. The high sensitivity obtained means a good performance can be expected 92% of the times, when attributing a good prognosis. The low specificity verified means 71% of the dogs would be wrongly given a poor prognosis but would actually end up surviving. But, while the consequences of a low specificity would be a closer monitoring of not so critically ill animals, if specificity was privileged over sensitivity the wrong attribution of a good prognosis to critically ill dogs could lead to a less rigorous clinical monitoring of these animals, risking their survival chances. Decision making methods, like this FFT, could help define clinical parameters and establish cut off values for them, making them a valuable tool in sepsis research.

4.5. Organ dysfunction (O)

The presence of organ systems dysfunction caused by a deleterious inflammatory response is what differentiates sepsis from an infection, and the presence of organ failure has been shown to correlate with the outcome. In the current study, no significant statistical association was found between organ dysfunction and outcome ($p = 0.1135$). Diverging from the results reported in other studies.

Kenney et al. (2010), on a veterinary medicine study on sepsis outcome prediction, reported that cardiovascular dysfunction, coagulation dysfunction, renal dysfunction ($p < 0.001$) and respiratory dysfunction ($p < 0.01$), were all independently associated with the outcome. On the same study it was also reported that mortality rate rose as the number of organ systems affected increased. Other variables reported to be independently associated with mortality on human medicine studies include blood pressure, altered coagulation times, serum creatinine, urinary output, need for vasopressor therapy, serum lactate and SOFA score (Granja et al. 2013; Rathour et al. 2015).

For the overall organ dysfunction score and its' correlation with the outcome, Rathour et al. (2015) described a good outcome prediction accuracy, with an AUC-ROC = 0.81. A significant statistical association between overall organ dysfunction score and outcome was also reported by Granja et al. (2013), with a $p < 0.001$.

The disparity between the results obtained and those abovementioned might reflect a poor correlation between the dysfunction taking place and the parameters chosen to characterize it, as the influence of each individual criteria on prognosis was not independently evaluated.

From the 72 dogs enrolled in this study, only 13 were considered to have some kind of organ dysfunction, each scoring 1. Hepatic lesion was the most frequent cause of organic alteration with 8 dogs falling under this classification, 6 due to hypoalbuminemia and 2 with elevated ALP. Of these 8 dogs 4 deceased and the other 4 survived. Hypoalbuminemia is a rather nonspecific parameter to access hepatic function especially during sepsis, as increased vascular permeability and shifting to acute phase protein production also contribute for the albumin decrease (Silverstein and Otto 2012; Drobatz et al. 2019). Considering that the population is composed by Parvovirus infected dogs, hypoalbuminemia can even be less specific as a hepatic function marker, with the most probable cause for hypoalbuminemia being gastrointestinal protein loss. Alkaline phosphatase is not directly related with impaired liver function, even though it can be elevated during sepsis secondary to cholestasis (Silverstein and Otto 2012). The ALP increment was slight in both dogs suggesting it could result from individual variation. The low specificity of the variables chosen to evaluate liver function and the reported inconsistency of hepatic dysfunction as a mortality predictor, could account for the results obtained on the present study.

From the remaining dogs 2 were considered to have renal dysfunction, one with a creatinine increment and the other with both creatinine and urea elevation. Only the first of these dogs died. Based on human medicine consensus the criteria that should be included in the definition of renal dysfunction include serum creatinine concentration, glomerular filtration rate and urine output. In the present study only creatinine and urea were considered as renal dysfunction markers, which could have impaired the ability to identify the presence of renal dysfunction on this sample (Bellomo et al. 2004). Nonetheless, Kenney et al. (2010) reported a strong association between renal dysfunction and outcome ($p < 0.05$) even when using serum creatinine concentration as the only renal dysfunction marker.

Finally, 3 dogs with low platelet count were included on the coagulation dysfunction group and they all survived. Even though coagulation dysfunction has been independently associated with mortality there are still conflicting results when it comes to platelet count. While Hauptman et al. (1997) reported thrombocytopenia as being a good sepsis marker ($p < 0.05$), Laforcade et al. (2009) reported no significant difference on platelet counts between dogs with sepsis and the control group ($p = 0.123$). The measurement of other coagulation markers like prothrombin time (PT), partial thromboplastin time (PTT), fibrin degradation products (FDP) and D-dimer (DD) concentrations, could have helped on the detection of more animals suffering from coagulation disorders, which could have helped improve the overall correlation between organ dysfunction and outcome.

None of the animals included showed signs of cardiovascular or respiratory dysfunction so it was impossible to assess their influence on the outcome. The fact that the criteria proposed to characterize both of these organ systems dysfunctions were rather subjective, as they require clinical judgment, could have accounted for the results obtained. Future studies should consider other cardiovascular and respiratory function markers. As far as the respiratory function is concerned there are, since 2007, veterinary medicine specific and consensual criteria to assess the presence of acute lung injury and ARDS. An animal must meet the first 4 criteria with the fifth criteria being a recommended but optional measure. The criteria are: acute onset (< 72h) of tachypnea or dyspnea; the presence of a known risk factor (which include systemic inflammatory response syndrome and sepsis); evidence of pulmonary capillary leak without increased pulmonary capillary pressure (shown by imaging or fluid sample collection); evidence of inefficient gas exchange; evidence of diffuse pulmonary inflammation (assessed through transtracheal wash or bronchoalveolar lavage) (Wilkings et al. 2007). Since none of the dogs had blood pressure registration the assessment of cardiovascular dysfunction by the need for vasopressor drug therapy is inevitably a subjective analysis, as peripheral pulse evaluation can vary between clinicians. Helpful clinical exams for the characterization of cardiac dysfunction could include echocardiography, as it would help identify the presence of biventricular dilatation or a decreased ejection fraction (Osterbur et al. 2014).

The low specificity of the parameters chosen to classify the organ dysfunctions, as well as the retrospective nature of the study that made impossible the inclusion of specific organ dysfunction tests and biomarkers that could help better characterize organic dysfunction, may be accountable for the results observed. Other classification systems like the SOFA can also be used to improve the scoring of organ dysfunction (O) and contribute to a more accurate overall PIRO scoring system.

4.6. PIRO

In the present study no significant statistical association was found between the total PIRO score and the outcome ($p = 0.093$). This result contrasts with what has been found on previous studies, where PIRO was found to have a good outcome prediction ability.

Multiple studies focused on the outcome prediction capacity of the PIRO and its' performance when compared with other classification systems. Even though different studies included distinct parameters and considered different classification criteria for each of the PIRO components, they all described a fair to good outcome prediction ability. One of the first studies to include one version of the PIRO score was Rubulotta

et al. (2009), which reported an area under the curve of 0.696. Even though this result defines only a fair mortality prediction ability for PIRO, it was equivalent to the AUC published for other scoring systems at the time, which ranged from 0.6 to 0.7. A similar discriminative power paired with an easier applicability makes PIRO, in the authors´ opinion, a good option when it comes to septic patients' stratification.

Howell et al. (2011) conducted a study that validated PIRO's utility. The proposed classification system was created based on independent variables found to be associated with mortality with statistic significance, which increased the outcome prediction accuracy. The scoring system was applied to a sample group and to two validation cohorts. Results revealed that mortality was strongly related to an increased PIRO score with an AUC-ROC of 0.9, 0.86 and 0.83, respectively. On a study conducted by Nguyen et al. (2012), PIRO performed better (AUC-ROC = 0.71) than MEDS and was comparable to the APACHE II. Li (2013) conducted a study on community acquired sepsis in which an AUC of 0.90 for PIRO 28-day mortality prediction was reported, outperforming the APACHE II. Granja et al. (2013) took on a Portuguese ICU-admitted community acquired sepsis population, and apart from reporting that each PIRO component was independently associated with mortality, also reported an AUC-ROC of 0.84 for the total PIRO outcome prediction accuracy. More recently, results reported by Songsangjinda and Khwannimit (2018) on septic patients admitted over a 9 year period, showed that the Moreno PIRO had the best discriminating capacity with an AUC-ROC of 0.835, outperforming all the other classification systems and only closely followed by SOFA (AUC-ROC = 0.828). One of the best discriminative capacity for PIRO mortality prediction was described by Rathour et al. (2015), with an AUC of 0.94. Overall, PIRO is considered a good system for the stratification of sepsis affected humans with an AUC-ROC approximately ranging from 0.7 to 0.9.

To our knowledge this is one of the first studies to propose PIRO classification criteria for animals and to evaluate their correlation with the outcome. Even though no significant statistical association was found between the total PIRO score and the outcome, this is the first version of the proposed model and can serve as reference for future studies.

On this sample the mortality rate was 18.1%, which may indicate an underrepresentation of the death group thus contributing to the results obtained, as sepsis mortality rates have been reported to go up to 68% and reaching 90% in the presence of septic shock (Bentley et al. 2007; Kenney et al. 2010). Even though canine parvovirus enteritis is a sepsis predisposing condition, limiting the inclusion criteria to a confirmed Parvovirus infection and not to a sepsis diagnosis, which would require the

fulfilment of the SIRS criteria plus an infection confirmation, may have biased the results by including non-septic dogs on the sample group.

The relatively narrow range of the total PIRO score may also have contributed to a worse outcome prediction capacity, this should be addressed in future studies which ought to include more parameters to better characterize the systemic inflammatory response and distinguish animals based on illness severity. The individual assessment of the parameters and how they independently affect the outcome, as well as the addition of other biomarkers that can help characterize the inflammatory response, could contribute to a better outcome prediction accuracy of the proposed PIRO scoring system.

5. Conclusion

The present study stands as a contribution for the development of a unanimously recognized and validated classification system for sepsis in dogs, thus helping in the disease characterization and stratification of septic animals. The validation of a classification system that could help in sepsis diagnosis, clinical decision making, therapy adjustments and population enrolment in future sepsis related studies, is the first step towards a better understanding of sepsis. To our knowledge this is the first study to propose and assess the implementation of a PIRO classification system in dogs, adapting it from what has been described in human medicine and testing it on a sepsis predisposed population of Parvovirus affected dogs.

Concerning the SIRS criteria this study confirms what has been their main criticism, a low specificity. While the SIRS 1991 criteria did not correlate with the outcome, when only considering animals with altered mucous membrane colour or CRT (SIRS 2001), thus increasing the specificity, a significant statistical association with the outcome was found ($p < 0.05$). Even though only considering animals upon mucous membrane or CRT changes may have induced a decrease in the criteria sensitivity, therefore resulting in the loss of truly septic dogs that did not meet the classification criteria, this comes in an attempt to give a response to the increasing need for more specific sepsis related systemic inflammatory criteria and contributes to the improvement of sepsis diagnosis in dogs.

The proposed PIRO classification system was not found to be a valid classification system for the population in question, as no significant statistical association was found between any of the PIRO´s elements and the outcome. Nevertheless, it comes to prove that it is possible to define and apply a classification system for sepsis susceptible animals is possible. Being one of the first versions of this model to be applied to animals could have accounted for the poor results found, when compared to the ones reported in human medicine studies. Additional adaptation based

on the what has been reported in this study is needed in order to improve its´s outcome prediction capacity and turn it into a useful tool in sepsis related matters. Further studies should be of a prospective nature and should consider including more and more specific variables, such as inflammation and organ dysfunction biomarkers, that could help characterize each of the PIRO´s components and consequently its´ overall performance.

Despite all the weaknesses verified, this study should provide the basis for future prospective studies in larger populations. Future studies should also account for the dynamic nature of sepsis and evaluate the sequential changes and the variation patterns during the hospitalization period and not only at admission.

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V - Appendices

Appendix 1. Fisher's exact test tables for predisposition (P)

	Death	Discharge
0-5	1	6
6-10	13	52

Appendix 2. Fisher's exact test tables for response (R)

	Death	Discharge
0-6	9	50
7-12	5	8

Appendix 3. Fisher's exact test tables for organ dysfunction (O)

	Death	Discharge
0	9	50
1	5	8

Appendix 4. Fisher's exact test tables for total PIRO

	Death	Discharge
0-10	1	19
11-20	13	39

Appendix 5. Fisher's exact test tables for SIRS 1991

SIRS 1991	Death	Discharge
Yes	11	36
No	3	22

Appendix 6. Fisher's exact test tables for SIRS 2001

SIRS 2001	Death	Discharge
Yes	8	14
No	6	44

Appendix 7. Article submitted to BMC Veterinary Research

Canine Parvovirus: a predicting canine model for sepsis

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Abstract

Background: Sepsis is a severe condition associated with high prevalence and mortality rates. Parvovirus enteritis is a predisposing factor for sepsis, as it promotes intestinal bacterial translocation and severe immunosuppression. This makes dogs infected by parvovirus a suitable study population as far as sepsis is concerned. The main objective of the present study was to evaluate the differences between two sets of SIRS (Systemic Inflammatory Response Syndrome) criteria in outcome prediction. The possibility of stratifying and classifying septic dogs using a proposed animal adapted PIRO

(Predisposition, Infection, Response and Organ dysfunction) scoring system was also assessed.

Results: The 72 dogs enrolled in this study were subjected to a score for each of the PIRO elements, except for the Infection, as all were considered to have the same infection score, and to two sets of SIRS criteria, in order to measure their correlation with the outcome. Concerning the SIRS criteria, it was found that the proposed alterations were significantly associated with the outcome (OR=4.09, $p<0.05$), contrasting with the original SIRS criteria ($p=0.352$) that did not correlate with the outcome. No significant statistical association was found between Predisposition ($p=1$), Response ($p=0.1135$), Organ dysfunction ($p=0.1135$), total PIRO score ($p=0.093$) and outcome.

Conclusion: These results suggest that increasing the SIRS criteria specificity may improve their prognostic value and their clinical usefulness. In order to improve the proposed PIRO scoring system outcome prediction ability, more specific criteria should be added, mainly inflammatory and organ dysfunction biomarkers.