UNIVERSIDADE DE LISBOA

FACULDADE DE MEDICINA VETERINÁRIA

SIRS IN FELINE PATIENTS – RISK FACTORS AND PROGNOSTIC FACTORS USING PANLEUKOPENIA AS A MODEL, IN A VETERINARY TEACHING HOSPITAL

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Drª Patrícia Carla Vieira Duarte

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SRIS EM PACIENTES FELINOS – FATORES DE RISCO E FATORES DE PROGNÓSTICO UTILIZANDO A PANLEUCOPÉNIA VIRAL COMO MODELO, NUM HOSPITAL ESCOLAR VETERINÁRIO

Resumo

A sépsis e a síndrome de resposta inflamatória sistémica (SRIS) são condições médicas graves, consideradas responsáveis por uma grande porção da morbilidade e mortalidade em ambiente hospitalar, tanto em animais como em humanos. Apesar de diversas tentativas de determinar critérios de diagnóstico de sépsis em animais e de desenvolvimento de novas abordagens terapêuticas, pouco já se concluiu sobre o assunto. A compreensão de potenciais fatores de risco para SRIS e sépsis em felinos, bem como de fatores de prognóstico para estes pacientes sépticos, é crucial para a identificação quase imediata de sépsis e para um maneio eficaz da condição.

Os objetivos do estudo foram testar dois conjuntos de critérios de diagnóstico para SRIS, identificar fatores de risco para SRIS em pacientes felinos e determinar fatores de prognóstico para os mesmos, de forma a possibilitar uma identificação mais rápida de gatos com panleucopénia em risco de SRIS e determinar um prognóstico mais realista.

Na primeira fase do estudo, foi avaliado um grupo de 70 gatos com diagnóstico positivo de panleucopénia viral felina, determinou-se a incidência de SRIS e foi realizada uma análise comparativa de forma a determinar a validade dos critérios SRIS de 1991 e 2001 para o diagnóstico de sépsis. Ambos os conjuntos de critérios mostraram associações estatisticamente significativas com o desfecho e poderão ser úteis em ambiente clínico, desde que apenas se considere SRIS se houver dois exames de estado geral alterados num período de 24 horas. De seguida, foi realizada uma análise uni e multivariada para identificar fatores de risco independentes para SRIS em gatos. Entre as variáveis avaliadas, foram considerados fatores de risco independentes para SRIS em gatos a presença de hipoalbuminémia (OR=3,2), hipoglicémia (OR=12,1) e linfopenia (OR=2,9) à admissão.

Na segunda fase do estudo, a amostra investigada foi de 25 gatos com diagnóstico positivo para SRIS, a partir da amostra inicial. Realizou-se uma análise univariada para determinar alguma associação entre variáveis e o desfecho (alta ou morte). Os resultados revelaram que o aparecimento ou manutenção de anorexia ou hiporréxia (OR=29,2), desidratação (OR=5,9) e estado mental alterado (OR=Inf), assim como a presença de linfopenia à admissão (OR=12,4), apresentavam uma associação estatisticamente significativa com o desfecho e foram considerados como fatores de prognóstico negativos para gatos com SRIS.

Estas descobertas contribuem para a compreensão e o maneio clínico de SRIS e sépsis em felinos, aprimorando a capacidade de identificar gatos em risco de desenvolver SRIS. Por outro lado, possibilita a determinação de um prognóstico realista nestes animais, com vista a gerir expectativas e proporcionar terapêuticas mais individualizadas.

Palavras-chave: Síndrome de resposta inflamatória sistémica; sépsis; panleucopénia felina; fatores de risco; fatores de prognóstico

SIRS IN FELINE PATIENTS – RISK FACTORS AND PROGNOSTIC FACTORS USING PANLEUKOPENIA AS A MODEL, IN A VETERINARY TEACHING HOSPITAL

Abstract

Sepsis and Systemic Inflammatory Response Syndrome (SIRS) are serious medical conditions, responsible for many deaths among animals and humans, and despite many attempts to determine diagnostic criteria for sepsis in animals and to develop new therapeutic approaches, few advances have been achieved. Understanding potential risk factors for SIRS and sepsis in feline patients, as well as prognostic factors, is crucial for effective management and prompt sepsis identification.

The objectives of this study were to test the efficacy of two different diagnostic criteria for SIRS, identify risk factors for SIRS in cats and determine potential prognostic factors for these patients. The study used cats diagnosed with feline panleukopenia virus infection as a model for sepsis, and was divided in two parts. The first part of the study included a cohort of 70 panleukopenia-positive cats and risk factors were determined for these patients. The second part of the study included 25 SIRS-positive cats from the original sample in order to identify potential prognostic factors for cats with SIRS.

In the first part of this study, a comparative analysis was performed using Fisher's exact test to determine the validity of the 1991 and 2001 SIRS criteria for the diagnosis of sepsis. Both criteria showed statistical significance ($p<0.05$) and may be useful in a clinical setting, provided there are two abnormal physical exams within a 24-hour period.

Next, univariate and multivariate analyses were performed to identify independent risk factors for SIRS. Chi-squared test and Fisher's exact test were used for the univariate analysis, and logistic regression was performed for multivariate analysis. Among the variables evaluated, the presence of hypoalbuminemia, hypoglycemia and lymphopenia upon admission were found to be independent risk factors for SIRS in cats ($p<0.05$), using panleukopenic cats as a model.

In the last part of the study, the focus was narrowed to the 25 SIRS-positive cats from the original cohort. Univariate analysis was performed using Fisher's exact test to determine whether any factors were associated with the outcome (survival to discharge or death). The results revealed that the maintenance or appearance of anorexia or hyporexia, dehydration and abnormal mental status during hospitalization, as well as the presence of lymphopenia on admission, were all statistically significantly associated with the outcome and were considered negative prognostic factors for cats with SIRS.

These findings contribute to the understanding and clinical management of feline SIRS, enhancing the ability to identify at-risk cats and define a realistic prognosis, in order to manage expectations and tailor strategies to further optimize patient care.

Keywords: Systemic Inflammatory Response Syndrome; sepsis; feline panleukopenia; risk factors; prognostic factors

SRIS EM PACIENTES FELINOS – FATORES DE RISCO E FATORES DE PROGNÓSTICO UTILIZANDO A PANLEUCOPÉNIA VIRAL COMO MODELO, NUM HOSPITAL ESCOLAR VETERINÁRIO

Resumo Alargado

A sépsis e a síndrome de resposta inflamatória sistémica (SRIS) são condições médicas graves e ainda são consideradas como responsáveis por uma grande porção da morbilidade e mortalidade em ambiente hospitalar, tanto em animais como em humanos, contando com mais de 5 milhões de mortes em humanos. É uma área muito estudada e investigada e, no entanto, em animais pouco já se concluiu sobre o diagnóstico, maneio e particularidades individuais de cada espécie, assim como potenciais tratamentos inovadores. Desde 1991, na primeira conferência global sobre sépsis, a comunidade médico-veterinária utiliza o conjunto de critérios SRIS para o diagnóstico de sépsis em animais, em conjunto com a confirmação de infeção. Ao longo do tempo, estes critérios foram sofrendo algumas adaptações, mas apesar de já terem sido descontinuados na medicina humana por serem considerados pouco específicos e sensíveis para este meio, continuam a ser utilizados em animais por falta de consenso na comunidade médico-veterinária, bem como da ausência de investigação com esse propósito. Desta forma, é crucial o desenvolvimento de novos critérios de diagnóstico ou melhoramento dos já presentes, bem como a identificação de potenciais fatores de risco para SRIS e sépsis em felinos, sendo esta espécie uma das menos estudadas neste âmbito. Para além disso, a identificação de fatores de prognóstico para estes pacientes sépticos também é essencial para a identificação quase imediata de sépsis, levando a uma abordagem mais individualizada e um maneio mais eficaz.

Os objetivos do estudo foram testar dois conjuntos de critérios de diagnóstico para SRIS, identificar fatores de risco para SRIS em pacientes felinos e determinar fatores de prognóstico para os mesmos. Foi realizado um estudo retrospetivo de coorte, utilizando a panleucopénia viral felina como modelo de SRIS, tendo sido dividido em duas partes com amostras diferentes. Na primeira parte deste estudo, foi utilizado um grupo de 70 gatos positivos para panleucopénia viral felina, e foi realizada uma análise comparativa com o auxílio do teste exato de Fisher de forma a determinar a validade dos critérios SRIS de 1991 e 2001 para o diagnóstico de sépsis. Os critérios de SRIS de 1991 incluem 4 parâmetros com certos valores a cumprir, específicos para gatos e cães. No caso dos gatos, apenas são considerados estando em SRIS se apresentarem 3 dos 4 seguintes parâmetros alterados: temperatura retal <37.8 ºC ou >39.7 ºC; frequência cardíaca <140 ou >225; frequência respiratória >40; leucócitos >19500/µL e/ou >5% leucócitos não segmentados. O conjunto de critérios de 2001 segue as sugestões da conferência, realizada nesse ano, de adicionar certos sinais clínicos ou resultados de análises ao conjunto de critérios de 1991. Assim, o conjunto SRIS de 2001 apenas aplica os parâmetros caso o animal apresente uma coloração alterada das membranas mucosas e/ou tempo de repleção capilar (TRC) alterado. Neste estudo, apenas se considerou SRIS caso o animal apresentasse 2 ou mais exames de estado geral alterados num período de 24 horas durante a sua hospitalização. Esta decisão foi tomada de forma a aumentar a especificidade dos conjuntos de critérios. Ambos os conjuntos foram

testados para avaliar alguma potencial associação com o desfecho e, segundo os resultados obtidos, ambos mostraram associações estatisticamente significativas com o desfecho (p<0,05). Assim, poderão ser úteis em ambiente clínico para o diagnóstico de SRIS, desde que apenas se considere SRIS se houver dois exames de estado geral alterados num período de 24 horas. Como o conjunto de critérios SRIS 2001 apresentou um valor de *p* inferior ao de 1991, para além de ter sido sugerido de forma a aumentar a especificidade, para o resto do estudo apenas os gatos positivos para o conjunto de critérios de 2001 foi considerado positivo para SRIS.

De seguida, foi realizada uma análise uni e multivariada para identificar fatores de risco independentes para SRIS em gatos, utilizando o mesmo grupo original de 70 gatos positivos para panleucopénia viral. Para a análise univariada, o teste de qui-quadrado e o teste exato de Fisher foram utilizados para testar a associação com o diagnóstico de SRIS (2001). Para esta análise univariada, foi utilizado um intervalo de confiança de 80% e, portanto, as variáveis que apresentavam valores de $p<0.2$ foram seguidamente inseridas no modelo de regressão logística para avaliar a independência de cada variável na análise multivariada. As variáveis que foram testadas no modelo de regressão logística foram as seguintes: peso ($p<0.01$), estado vacinal ($p<0.2$), realização de transfusão de produtos sanguíneos ($p<0.2$), presença de hipoalbuminémia à admissão ($p<0.05$), hipoproteinémia à admissão ($p<0.05$), hipoglicémia à admissão ($p<0.0001$), linfopenia à admissão ($p<0.1$) e valor do hematócrito à admissão ($p<0.2$). Entre as variáveis avaliadas, a presença de hipoalbuminémia, hipoglicémia e linfopenia à admissão foram considerados fatores de risco independentes para SRIS em gatos ($p<0,05$), utilizando gatos com panleucopénia viral como modelo. O peso foi incluído no modelo multivariado, mas deixou de se mostrar estatisticamente significativo e sendo assim, não foi considerado como fator de risco independente. A presença de hipoproteinémia à admissão apenas foi eliminado do modelo de regressão logística pela natural associação entre os valores de proteínas totais com os valores de albumina séricos, sendo assim fatores confundidores. As restantes variáveis foram descartadas do modelo de regressão logística por variados motivos, entre os quais a perda de significância estatística ao intervalo de confiança escolhido (95%) ou presença de fatores co-dependentes que poderão ter influenciado o resultado inicial na análise univariada.

Posteriormente, na última fase do estudo, o foco foi reduzido apenas para os 25 gatos com diagnóstico positivo para SRIS do conjunto original de 70. Nesta fase, muitas outras variáveis foram analisadas, como certos sinais clínicos ou medicações que terão sido realizadas. Foi feita uma caracterização de SRIS felina e, posteriormente, foi realizada uma análise univariada para determinar se alguma variável estava estatisticamente associada com o desfecho (alta ou morte) e, desta forma, determinar potenciais fatores de prognóstico nestes pacientes. A análise univariada foi realizada com o auxílio do teste exato de Fisher, com um intervalo de confiança de 95%. Os resultados revelaram que o aparecimento ou manutenção de anorexia ou hiporréxia durante a hospitalização e o aparecimento ou manutenção de desidratação e estado mental alterado durante a hospitalização, assim como a presença de linfopenia à admissão, apresentavam uma associação estatisticamente relevante com o

desfecho (morte) e foram considerados como fatores de prognóstico negativos para gatos com SRIS. Devido ao número reduzido de casos na amostra, não foi possível realizar uma análise multivariada por regressão logística.

As descobertas deste estudo poderão contribuir para a compreensão e maneio clínico de SRIS e sépsis em pacientes felinos, sendo que esta espécie é das menos estudadas neste âmbito. Desta forma, foi possível determinar alguns fatores de risco ou indicadores de SRIS em gatos, aprimorando assim a capacidade de identificar gatos em risco de desenvolver SRIS e sépsis. Por outro lado, a determinação de fatores de prognóstico possibilitou a determinação de um prognóstico mais preciso em certos casos, de forma a gerir espectativas e eventualmente proporcionar terapêuticas e abordagens mais individualizadas para os diferentes pacientes.

Palavras-chave: Síndrome de resposta inflamatória sistémica; sépsis; panleucopénia felina; fatores de risco; fatores de prognóstico

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4. Shapiro-Wilk Normality test of “Age” in 25 SIRS positive cats
5. Abstract submitted to the 48th World Small Animal Veterinary Association (WSAVA) World Congress – “SIRS in Feline Patients – Identification of Risk and Prognostic Factors using Viral Panleukopenia as a model”

Abbreviations and Acronyms:

% – percentage

> – superior

< – inferior

ADH – antidiuretic hormone

ALT – alanine transaminase

ALP – alkaline phosphatase

ARDS – Acute respiratory distress syndrome

AST – aspartate aminotransferase

aPTT – activated partial thromboplastin time

ATP – adenosine triphosphate

CBC – complete blood count

CI – confidence interval

CIRCI – critical illness-related corticoid insufficiency

CK – creatine kinase

CPV – canine parvovirus

CRT – capillary refill time

CT – computerized tomography

DAMP - damage-associated molecular patterns

DIC – Disseminated intravascular coagulation

DNA – deoxyribonucleic acid

EC – erythrocyte concentrate

EGDT – early goal-directed therapy

ELISA – enzyme-linked immunosorbent assay

FeLV – Feline Leukemia Virus

FFP – fresh frozen plasma

FP – feline panleukopenia

FPV – feline panleukopenia virus

URTD – Upper respiratory tract disease

GGT - γ-glutamyltransferase

H - hour

HES – hydroxyethyl starch

HIV – human immunodeficiency virus

HMGB1 – high mobility group box 1

ICU – Intensive Care Unit

IFN – interferon

IL – Interleukin

IVIG – intravenous immunoglobulins

KCl – potassium chloride

Kg - kilogram

LR – Ringer's lactate

LV – left ventricle

Mcg – microgram

MDA – maternally-derived antibodies

Min - minute

miRNA – microRNA

ml – milliliters

MLV – modified live vaccines

mmHg – millimeter of mercury

MOD – multiple organ dysfunction

MODS – multiple organ dysfunction syndrome

MRI – magnetic resonance imaging

NaCl – sodium chloride

NET – neutrophil extracellular trap

NK – natural killer

NO – nitric oxide

NOD-like - nucleotide-binding oligomerization domain

PAMP – pathogen-associated molecular patterns

PCR – polymerase chain reaction

PD-1 – programmed cell death-1

PRR – pattern recognition receptors

PT – prothrombin time

qSOFA – quick Sequential Organ Failure Assessment

RBC – red blood cell

rFeINF – recombinant feline interferon omega

RIG - retinoic acid-inducible gene-1

RLR – RIG-like receptors

RNA – ribonucleic acid

ROS – reactive oxygen species

SAE – sepsis associated encephalopathy

SSC – Surviving Sepsis Campaign

Si-AKI – sepsis-induced acute kidney injury

SCCM – Society of Critical Care Medicine

SIRS – Systemic Inflammatory Response Syndrome

SOFA – Sequential Organ Failure Assessment

TLR – Toll-like receptors

TNF – Tumor necrosis factor

VLDL – very-low-density lipoproteins

1. Curricular Traineeship Report

The curricular traineeship occurred between 3rd of January and 29th of April of 2022 at the Veterinary Hospital of Berna in Lisbon, and between 6th of June and 3rd of September 2022 at the Isolation and Biological Containment Unit, University of Lisbon's Faculty of Veterinary Medicine.

1.1. Veterinary Hospital of Berna, Lisbon (HVB)

The internship that occurred at HVB was divided into four rotations, these being Inpatient care, Surgery, Imagiology and Consultations in various areas of expertise, with a total of 696 hours (198 h in Imagiology, 111 h in consultations, 126 h in inpatient care and 189 h in surgery, having also done 72 hours of emergency consultations, procedures and hospital admittance). These will be individually approached.

1.1.1. Imagiology Rotation

The imagiology rotation consisted in accompanying the Hospital's sonographer in all echographies that were scheduled and performed, as well as preparation of the animal for the echography to take place (placement, trichotomy, alcohol and gel application, and, if needed, catheter placement and mild sedation). Experience was gained in identifying most abdominal organs and recognizing abnormalities. Most ultrasonographies that took place were abdominal and, in some cases, thoracic ultrasonographies and echocardiograms were also performed. When needed, fine needle aspiration was performed to the liver, spleen, lymph nodes and neoplastic masses, and cystocentesis was commonly executed. It was possible to assist and participate in four thoracocenteses and one pericardiocentesis. These procedures would be carried out in both inhouse patients as well as animals that were accompanied in other veterinary clinics or hospitals that had been referred to us. During the imagiology rotations, I assisted in more than 110 ultrasounds, and was able to perform 22 ultrasounds and 3 cystocenteses.

Other than the ultrasounds, it was possible to assist in other imagiologic procedures, namely computerized tomography scans and radiographies. The computerized tomography scans were performed with some regularity and the preparation of the animal included catheter placement, pre-medication and sedation, application of intravenous fluid therapy, placement of the animal on the tomography table, intubation and ventilation, placement of pulse oximeter, assessment of the patients' vital parameters during the scan, and once the procedure was completed, extubation and assessment of the patient during its recovery. As for the radiographies, it was possible to assist in many different radiographies of both cats and dogs, including abdominal, thoracic and orthopedic. When needed, sedation would take place and barium sulfate was administered orally to one patient for a contrasted x-ray. Many of the x-rays were of oncologic nature, so identification of metastases was very common.

1.1.2. Surgery Rotation

The surgery rotation consisted in accompanying and participating in various surgeries throughout the week, as well as emergency surgeries during the weekend. It was possible to observe and take part in many interesting surgeries, such as oncological surgery with removal of masses from the skin, muscle and different organs or removal of portions of metastasized organs. Moreover, it was possible to assist in other interventions, such as digit and member amputation, 1 tail amputation, 1 ear amputation, orthopedic surgeries, cystotomies and biopsies to the liver (manual or “Tru-Cut”), spleen, gastrointestinal tract, bladder and kidney. It was possible to assist in elective sterilizations, which were common in both cats and dogs, male and female, as well as in cases with pyometra. Electrochemotherapy was a common procedure, especially in cats with squamous cell carcinoma. Lastly, it was possible to assist in many endoscopies and one rhinoscopy, with collection of biopsy samples.

1.1.3. Inpatient care

During the inpatient care rotation, it was possible to accompany and follow the development and progression of the patients that were admitted, as well as participate in the discussion of the clinical cases that occurred many times throughout the day. I was able to participate in the patients’ physical exams, preparation and administration of medication (oral, subcutaneous, intramuscular, intravenous, and ocular), intravenous catheter placement and fluid therapy administration, as well as urethral catheterization, wound cleaning and dressing, bandage application, bone fracture stabilization, enemas, among others. It was possible to assist in some emergency procedures, such as gastric torsion in a dog and resuscitation. Most patients were considered internal medicine or oncology cases, but there were some animals admitted to the Infectious ward with infectious agents. Most of these infectious disease patients were, however, referred to other hospitals in order to diminish the probability of transmitting the disease to the oncology patients. Among these, the observed diseases were feline infectious peritonitis, feline panleukopenia, calicivirus suspects, canine parvovirus and FIV.

1.1.4. Consultations

During the consultation rotations, it was possible to accompany and assist in consultations of many areas of expertise. When needed, specialized veterinarians in certain fields would come in. As such, it was possible to assist in consultations of dermatology, cardiology, internal medicine, ophthalmology, orthopedia and general practice. Assistance in these consultations would translate into anamnesis collection, performing physical exams, collecting samples and analyzing them (blood, urine, skin), vaccine administration, aiding in fine needle aspiration of small skin nodules, preparing medications, wound disinfection and bandaging, among others. As for euthanasia, it was possible to assist in its execution.

1.1.5. Oncology

The Veterinary Hospital of Berna in Lisbon is an oncology reference hospital, where a sizeable portion of the casuistic is of the oncologic nature. Therefore, throughout the internship, there was follow-up of oncologic patients during the whole process of diagnosis, individual therapeutic protocol development, and treatment (resorting to chemotherapy, electrochemotherapy, immunotherapy and oncologic surgery) and it was possible to observe many patient recoveries. Malignancies of all types were observed, such as melanomas, squamous cell carcinomas, mast cell tumors, lymphomas, osteosarcomas and even pulmonary mastocitomas. Many notions regarding chemotherapy, immunotherapy and safety issues were introduced.

1.2. Isolation and Biological Containment Unit (UICB), Faculty of Veterinary Medicine of the University of Lisbon

The second portion of my curricular traineeship occurred in the Isolation and Biological Containment Unit of the Faculty of Veterinary Medicine. The traineeship was 3-months long, where I was able to aid and assist in patient diagnosis, treatment, and recovery. The trainees had certain tasks and responsibilities, such as cleaning and disinfecting the premises every morning, prepare medication and food for the patients, collect biological samples for the biobank and for further analyses, aid and perform physical examinations, record all physical examination findings in the clinical files, administrate medication, among others. It was possible to assist in first vaccination consultations as well as infectious disease consultations and respective follow-ups. During this period, it was possible to accompany the patient's evolution and assist the veterinary professionals in decision making and participate in the daily case discussion, as well as assist in many interventions such as nasoesophageal tube placement, ultrasonographies, blood product transfusions, catheter placement, among others. All of the referred activities were performed under supervision of Professor Solange Gil and Dr. Inês Machado. I had the opportunity to observe patients with certain infectious diseases, such as feline panleukopenia, canine parvovirosis, FIV, FeLV, calicivirus, herpesvirus, multidrug-resistant infections and even infectious canine hepatitis. During this internship, it was possible to access the clinical archives to obtain the necessary data to accomplish this study.

1

2. Literature Review

2.1. Systemic Inflammatory Response Syndrome and Sepsis

2.1.1. General Notions and SIRS

Throughout its history, the definition of sepsis has undergone several revisions and updates. Presently, it is understood to be a severe medical condition characterized by life-threatening organ dysfunction caused by a dysregulated host response to infection, a definition proposed by a task force at the Society of Critical Care Medicine's (SCCM) 45th Critical Care Congress, held in 2016 in Orlando, Florida (Singer et al. 2016). Prior to that Conference, sepsis was diagnosed when a human patient fulfilled 2 out of 4 systemic inflammatory response syndrome (SIRS) criteria, along with the confirmation of infection. It was then noted that these criteria, albeit somewhat sensitive, lack specificity for organ dysfunction and have, therefore, been put aside as sepsis-defining criteria in humans. As such, and according to the consensus reached, sepsis is determined when infection is present or suspected in conjunction with evidence of organ dysfunction, established by the sequential organ failure assessment (SOFA) score and the quick SOFA (qSOFA) score. Septic shock is considered when the circulatory and cellular/metabolic abnormalities are severe enough to increase mortality (Singer et al. 2016). This may be defined by sepsis -induced hypotension refractory to fluid resuscitation, requiring vasopressors to maintain systolic blood pressure >90 mmHg or mean arterial pressure >70 mmHg, in conjunction with hyperlactatemia (<2mmol/L) (Singer et al. 2016)

Nonetheless, the SIRS criteria continue to be currently employed in the diagnosis of sepsis in dogs and cats, given the lack of consensus within the veterinary medical community regarding a potential modification in the clinical identification of sepsis (Montealegre and Lyons 2021). Systemic Inflammatory response syndrome is defined by the systemic inflammatory response to a variety of severe clinical insults (Bone et al. 1992). Criteria to determine onset of this inflammatory response include four parameters with certain cut-off values (Table 1), and cats are diagnosed with sepsis when at least 3 out of the 4 following criteria are fulfilled, in addition to infection confirmation: body temperature <37.8 ºC or >39.7 ºC; heart rate <140 or >225 beats per minute (bpm); respiratory rate of >40 breaths per minute; leucocytes >19,500/µL and/or >5% band neutrophils. Dogs are considered as septic when 2 out of 4 of the SIRS criteria are met (Sykes and Epstein 2013). These criteria were evaluated both in cats and dogs and have been used in clinical practice ever since (Hauptman et al. 1997; Brady et al. 2000).

In humans, sepsis remains as one of the most important causes of morbidity and mortality, accounting for more than 30 million sepsis diagnoses and more than 5 million deaths per year (Brent 2017). In veterinary medicine, however, sepsis prevalence is not a well-documented phenomenon, due to its complex diagnosis, and much less is known about its

epidemiology (Babyak and Sharp 2016). Some studies show, nonetheless, that mortality rates for cats with sepsis can range from 33 to 79% (Sergeef et al. 2004; Parsons et al. 2009; DeClue et al. 2011; Babyak and Sharp 2016). Therefore, it appears likely that sepsis accounts for a large portion of deaths both in dogs and cats in the intensive care unit (ICU) environment (Babyak and Sharp 2016; Montealegre and Lyons 2021).

Table 1 – Systemic Inflammatory Response Syndrome (SIRS) criteria for dogs and cats (adapted from Sykes and Epstein 2013)

Criteria	Dog	Cat
Heart Rate	>140 bpm	<140 or >225 bpm
Respiratory Rate	>30 breaths/min or PCO_2<32 mmHg	>40 breaths/min
Temperature	<37.8 or >39.4 ºC	<37.8 or >39.7 ºC
Leukocyte count	<6000 or >16000 cells/µL or >3% band neutrophils	>19500 cells/µL or >5% band neutrophils

Legend: % - percent; ºC – degrees Celsius; bpm – beats per minute; cells/µL – cells per microliter; mmHg – milimeter of mercury; min – minute; SIRS – Systemic Inflammatory Response Syndrome

2.1.2. Pathogenesis

2.1.2.1. Initiation of an Immune Response

The immune system is a complex network of cells, cytokines and factors that intertwine and collaborate among each other in order to protect the host and develop a response against the invading pathogen. In a first instance, there is activation of the innate immune response to the insult via the recognition of pathogen components by specialized receptors that are expressed by many effector immune cells and also epithelial cells, such as intestinal epithelial cells and other mucosa. These receptors are germline-encoded and named pattern recognition receptors (PRRs), and are usually found in the subcellular and extracellular compartments of neutrophils, macrophages, dendritic cells, lymphocytes, platelets, natural killer (NK) cells and fibroblasts (Janeway 1989; Kumar et al. 2011). PRRs have the ability to recognize specific repetitive molecular structures that are frequently found on and produced by microorganisms, both pathogenic as well as non-pathogenic, and that are called pathogen-associated molecular patterns (PAMPs). These include bacterial lipopolysaccharide, peptidoglycan and lipoteichoic acids, fungal β-glucan, and viral nucleic acids (Medzhitov 2007). Besides identifying potentially threatening pathogens, PRRs also bind to patterns that are products of host cell damage and indicate tissue and organ lesion, such as intracellular ATP or mitochondrial DNA (Matzinger 1994; Tang et al. 2012). These damage-associated molecular patterns (DAMPs) act as PAMPs in the activation of innate immune cells and initiation of the host immune response.

There are four major PRR families: Toll-like receptors (TLR), C-type lectin receptors, NOD-like receptors (nucleotide-binding oligomerization domain) and retinoic acid-inducible gene-1 (RIG) like receptors (RLR). These PRRs can be categorized in transmembrane PRRs, which detect extracellular PAMPS, or cytosolic PRRs which are found in phagosomes or endosomes. Independent of their location, all PRRs can identify microorganisms and activate an appropriate innate immune response (Palm and Medzhitov 2009).

PRR activation initiates a series of reactions, both proinflammatory and anti-inflammatory, that help develop the immune response to the insult and prevent the spread of infection. Activation of intracellular signaling pathways leads to multi-gene expression and a transcriptional response, culminating in production of proinflammatory cytokines, tumor necrosis factor (TNF) and interferon (IFN), which is an essential step for innate and adaptive immune responses to occur (Brubaker et al. 2015; Gyawali et al. 2019; Montealegre and Lyons 2021). If kept under control, the inflammatory response plays a crucial role in the activation of an immune response, as it indicates the presence of a pathogen and allows a rapid mobilization of innate cells to the injury site. However, if the inflammatory response becomes dysregulated there can be nefarious consequences and sequela, much of which are demonstrated in a septic shock scenario (Montealegre et al, 2021).

2.1.2.2. Local and Systemic Inflammatory Response

Subsequent to host-pathogen interactions via PRR ligation, many intracellular signalling transduction pathways are activated culminating in multi-gene expression that lead to adaptive immune responses and inflammation (Medzhitov 2007; Montealegre and Lyons 2021). The transcription of genes upon command results in the release of proinflammatory cytokines, including IL-1β, IL-6 and TNF-α (Medzhitov 2007; Gyawali et al. 2019). Activation of some NOD-like receptors may also promote aggregation into large multiprotein intracellular complexes, the inflammasomes, which control the activation of caspases and secretion of caspase-1-dependent cytokines like IL-1β and IL-18 (Broz and Dixit 2016; Gyawali et al. 2019; Montealegre and Lyons 2021).

Neutrophils, for instance, are able to mount effector mechanisms against pathogens such as production of reactive oxygen species (ROS), phagocytosis, cell-death with proteases and launch of neutrophil extracellular traps (NETs), which aid in pathogen clearance. Formation of excessive NETs, on the other hand, promotes inflammation and endothelial and tissue lesion in sepsis (Camicia et al. 2014; Montealegre and Lyons 2021)

Exosomes also amplify the inflammatory response, as the multiple cytokines they carry, such as IL-1α, IL-1β, IL-6, IL-18, as well as miRNAs, DAMPs and NETs, mediate proinflammatory effects and activate neutrophils, macrophages and endothelial cells.

Macrophages, on the other hand, release TNF-α and IL-6 that contribute to vascular dilation, vascular endothelial dysfunction and leakage (Montealegre and Lyons 2021).

All these proinflammatory cytokines stimulate leukocyte activation and proliferation, as well as activation of the complement system (Gyawali et al. 2019). They also upregulate the expression of endothelial adhesion molecules and chemokines, promote tissue factor production, and induce hepatic acute phase reactants (Hotchkiss et al. 2016; Gyawali et al. 2019). However, during sepsis, this immune response is heightened, leading to consequential tissue damage and host cell death.

2.1.2.3. Sepsis and Septic Shock

During the development of an immune response, the excessive production and secretion of many proinflammatory cytokines may lead to the formation of a cytokine storm (Tisoncik et al. 2012). This phenomenon is characterized by large quantities of circulating proinflammatory cytokines, produced and released by innate immune cells. Almost simultaneously, there is release of anti-inflammatory cytokines such as IL-10, as well as a diminished inflammatory cytokine production by monocytes, macrophages and dendritic cells, in an attempt to control the inflammation (Tamayo et al. 2011; Tisoncik et al. 2012). The development of the cytokine storm demonstrates part of the dysregulation of the immune system that occurs during SIRS and sepsis.

Sepsis is considered when the localized infection is spread and affects normal tissue distant from the injury site (Nathens and Marshall 1996), and is characterized by two simultaneous states, a hyperinflammatory state as well as its compensatory hypoinflammatory answer, due to a prior innate and adaptive immune dysfunction (Jarczak et al. 2021). It is not well known how the transition is made, and while there are studies that show that dogs with ongoing sepsis have both these phases present, other studies in cats show that there is occurrence of a very short or inexistent hyperdynamic phase, thus predominating the hypoinflammatory state characterized by hypothermia, vasoconstriction with pale mucous membranes and bradycardia (Brady et al. 2000; Greiner et al. 2008; Costello 2015).

The later part of sepsis and initiation of septic shock is characterized by a more dominant hypoinflammatory and immunosuppressive state. Many factors contribute to this immunosuppression, such as a dysregulated anti-inflammatory response, a dysregulated expansion of T-regulatory cells, lower response to cytokines, T and B cell apoptosis with lymphocyte exhaustion, as well as the occurrence of immunoparalysis (Hotchkiss, Tinsley, et al. 2001; Remick 2007; Berlot and Passero 2016). This phenomenon is described as a trigger-less state, where there is diminished capacity of an immune response to a pathogen. This is due to many determinants such as macrophage deactivation, an increase in anti-inflammatory cytokines, expression of checkpoint inhibitors like programmed cell death-1 (PD-1), increased

suppression to immune cells, B and T cell impairment and cell anergy (Hotchkiss et al. 2001; Remick 2007; Berlot and Passero 2019).

2.1.2.4. Homeostasis Dysregulation

During sepsis, there is simultaneous activation of the inflammatory as well as the coagulation cascades. The dysregulation that occurs in the coagulation process is thought to be multifactorial, where both the complement system as well as certain cells like neutrophils and platelets play a pivotal role. A dysregulated activation of the complement cascade promotes excessive activation of the membrane attack complex (MAC), a lytic component of the complement (Kerr and Richards 2012). This causes injury of endothelial cells which, consequently, secrete tissue factor, a protein with procoagulant properties. Tissue factor may also be released by macrophages and neutrophils in response to pathogen recognition, and leads to platelet activation and production of thrombin, thereby triggering microthrombi formation (Hotchkiss et al. 2016). These microthrombi may severely impact local perfusion, resulting in tissue hypoxia and potentially leading to multiple organ dysfunction (MOD). An excessive production of NETs by neutrophils also potentiates the coagulation cascade. Antithrombin and activated protein C down-regulation additionally contributes to the hypercoagulable state, as their anticoagulant properties physiologically mediate coagulation (Esmon 2003; Hotchkiss et al. 2016; Silverstein and Otto 2023). Activated protein C inhibits procoagulant factors Va and VIIIa and, without degradation of these factors as well as of TNF-α and IL-1β, these will all be in excess and contribute further to the maintenance of the hypercoagulable state and potentiate microvascular thrombi formation (Silverstein and Otto 2023). On the other hand, uncontrolled bleeding can occur as a consequence of depletion of clotting factors and consumptive thrombocytopenia (Levi et al. 2013; Hotchkiss et al. 2016), and the hypercoagulable state may transition to disseminated intravascular coagulation (DIC). This is characterized by thrombocytopenia, increased fibrin degradation products derived from fibrinolysis and exhaustion of prothrombin, fibrinogen and factor X and V reserves (Hotchkiss et al. 2016).

This intersection between inflammation and the coagulation cascade can have nefarious consequences, such as disseminated intravascular coagulation (DIC), cytotoxic and ischemic MOD and death. This phenomenon is called immunothrombosis, where a dysfunctional activation of platelets as well as neutrophils and their excess production of NETs induce a hypercoagulable state (Gyawali et al. 2019). The occurrence of intravascular coagulation and microvascular occlusion by microthrombi leads to hypoperfusion, hypoxia and, eventually, cytotoxic and ischemic multiple organ dysfunction (Hotchkiss et al. 2016).

2.1.2.5. Cellular, Tissue and Organ Dysfunction

This tissue hypoperfusion ultimately causes a decrease in oxygen delivery and thus decreased utilization by cells. It is thought that this is the main underlying mechanism which leads to tissue and organ dysfunction. Additionally, an increased production of lactic acid due to the cellular anaerobic glycolysis takes place and a drop in ATP levels caused by mitochondrial dysfunction may damage tissue at a cellular level. This occurrence in different tissues leads to specific organ systems dysfunction which are collectively responsible for SIRS and sepsis morbidity and mortality (Gyawali et al. 2019). These will be individually approached further on.

2.1.3. Risk Factors for SIRS and Sepsis

Risk factors for sepsis can encompass risk factors for infection and risk factors for organ dysfunction upon infection, the latter being less understood (Mayr et al. 2014; Cecconi et al. 2018). As sepsis is a syndrome that can develop from many different diseases, the identification of risk factors is a complex task, and may be influenced by individual factors associated with the pathophysiology of each infection.

In humans, risk factors for neonatal sepsis have been investigated and some studies mention mainly peripartum issues that influence sepsis onset, such as chorioamnionitis (Yancey et al. 1996; Ershad et al. 2019; Odabasi and Bulbul 2020). In human sepsis, some factors are considered as certain populations are at a higher risk of developing infection. Examples of these are age, where sepsis occurs mainly in patients which are over 67 years old, gender and race (Combes et al. 2009; Iskander et al. 2013; Mayr et al. 2014). Many studies have demonstrated that men demonstrate a higher incidence of sepsis and SIRS than women, although other reports show opposite results (Drechsler et al. 2012; Angele et al. 2014). Other risk factors may be chronic treatment with immunosuppressants or immunosuppressive conditions such as human immunodeficiency virus (HIV) infection, cancer, diabetes and obesity, malnutrition, alcohol abuse, prosthetic devices and residence in long-term facilities (Iskander et al. 2013; Mayr et al. 2014; Huson et al. 2015; Cecconi et al. 2018). However, it is not fully understood why some patients have a different immune response in comparison to others, meaning that they are more prone to develop organ dysfunction, although it is suspected that genetic factors might have a more important role than the presence of cardiovascular disease (Sorensen et al. 1988; Marshall 2006; Mayr et al. 2014; Cecconi et al. 2018). Host genetics may be both a risk factor for infection as well as a risk factor for further organ dysfunction and septic shock. Multiple genes and their interactions with the environment are likely required for the onset of sepsis (Cecconi et al. 2018). Some studies have identified certain polymorphisms in specific factors, such as TNF-α and Toll-like receptors, and their influence in sepsis.

Currently, there are few reports of risk factors for SIRS or sepsis in feline patients. As mentioned before, most risk factors depend on the infection site, its pathophysiology and on the different interventions that may promote an inflammatory state. Some of the risk factors for sepsis in cats that are mentioned in the literature are the complications of certain diseases, such as pyothorax, pneumonia, septic peritonitis, bacteremia secondary to gastrointestinal disease, endocarditis, pyelonephritis, pyometra, osteomyelitis and bite wounds (Silverstein and Otto 2023). Bacteremia secondary to gastrointestinal disease may occur due to disruption of gut epithelial cells and increased permeability of the intestinal barrier, with bacterial translocation from the intestinal lumen into the bloodstream. However, risk factors for onset of organ dysfunction and susceptibility for SIRS are less investigated.

One study conducted by Deitschel et al. (2010) observed an altered anti-inflammatory mediator production, namely of IL-10, in older dogs, suggesting age-associated changes in the immune response that may promote an aggravated proinflammatory state. However, these findings have not been reported in cats. As for breed, its influence on the inflammatory state has been studied mainly in dogs, where parvoviral enteritis susceptible breeds produced increased values of TNF-α, suggesting that breed could be a predisposing factor for cytokine production variations that could impact the inflammatory response (Nemzek et al. 2007).

In one study which evaluated cats with pyothorax, indoor-outdoor environment and multicat houses were considered as risk factors for sepsis (Waddell et al. 2002). Another study investigating neonatal feline sepsis determined that factors that predispose kittens to septicemic conditions induced by natural microflora include inadequate thermoregulation and nutrition, viral infections, parasitism and immune system defects (Hoskins 1993). A different study conducted by Grimes et al. (2011) investigated septic risk factors in a gastrointestinal post-operative scenario in dogs, which included intraoperative hypotension, post-operative hypoalbuminemia and hypoproteinemia as risk factors (Grimes et al. 2011). In cats, a recent study reported an association between elevated serum amyloid A (SAA) values, hyperbilirubinemia and presence of toxic neutrophils and sepsis diagnosis (Troìa et al. 2017). These findings, however, may be viewed in septic cats as a consequence of the onset of sepsis as opposed to being risk factors, thus being potential biomarkers for diagnosis is cats.

2.1.4. Prognostic Factors for SIRS and Sepsis in Cats

In cats with sepsis, mortality rates vary between 33 and 79%, despite proper management and treatment (Sergeeff et al. 2004; Parsons et al. 2009; DeClue et al. 2011; Babyak and Sharp 2016) and do not appear to differ much from mortality rates of cats with non-infectious SIRS (King 1994; DeClue et al. 2011; Babyak and Sharp 2016). Several attempts have been made to define prognostic factors for SIRS and sepsis in cats and dogs and a few studies have reported some, although most prognostic and diagnostic studies in

cats are based on small samples (Gori et al. 2021). The search for and identification of new prognostic factors for sepsis in cats is therefore essential for clinicians to watch for certain predictive clinical signs and blood tests that may indicate a worse outcome and adapt treatment and management.

Of the many different prognostic scoring systems created for human patients, some have been adapted for veterinary use, such as APACHE, PIRO, SOFA, qSOFA and APPLE (Hayes et al. 2011). The SOFA and qSOFA score have substituted the SIRS criteria for diagnosing human sepsis (Singer et al. 2016). However, this has not yet been adapted in veterinary medicine and studies are needed to evaluate whether veterinarians can extrapolate these findings. Only two recent studies evaluated predictive scoring using the qSOFA score in cats. One evaluated critically ill cats and dogs and only reported statistical association with the outcome 4 hours after admission and sensitivity of around 80% (Ferreira 2022). The other study evaluated feline patients with panleukopenia infection, and determined that although qSOFA was insufficient to predict sepsis, it may hold prognostic value when interpreted in conjunction with hematological results (Gülersoy et al. 2023). Feline APPLE score is a stratification system for hospitalized cats, although it shows no predictive or prognostic value (Hayes et al. 2011). Other work also attempted an adaptation of the PIRO score for septic dogs, with results showing no significant statistical association with the outcome (Alves et al. 2020).

Sepsis in human patients is nowadays defined as a life-threatening organ dysfunction due to a dysregulated host response to infection (Singer et al. 2016). As such, evidence of organ dysfunction should be expected to be associated with the outcome. This evaluation has been made in cats, where the presence of multiple organ dysfunction syndrome (MODS) at presentation increases the risk of death (Troìa et al. 2019). Troìa et al. (2019) reported that the increased number of dysfunctional organs was a strong predictor of mortality, as previously reported in humans (Nfor et al. 2006; Osterbur et al. 2014). Individually, the presence of sepsis-associated acute kidney injury (sa-AKI) and cardiac dysfunction in cats were associated with negative outcomes, whilst respiratory, hepatic and hemostatic dysfunction were not (Troìa et al. 2019). Conversely, a study evaluating MODS in dogs determined that respiratory, cardiac, renal and hemostatic dysfunction were associated with a negative outcome (Kenney et al. 2010). These findings should help adapt the SOFA score to veterinary medicine as possible future criteria to determine sepsis in animals.

Studies evaluating clinical signs or laboratory analyses as prognostic indicators of sepsis in cats are lacking, and having only one or a few reports on each clinical sign or test is insufficient to establish solid criteria. These include:

- Body temperature; in one study it was reported no association between hypothermia or fever and negative outcome (Scotti et al. 2019).

- Mental status; one study evaluated mentation in cats with FP for qSOFA evaluation purposes, and determined that septic cats demonstrated more abnormal mentation status prevalence than healthy cats, and that together with other hematological results may serve as a prognostic factor (Gülersoy et al. 2023). Moreover, it has been demonstrated that human septic patients with neurological dysfunction have poorer prognosis (Osterbur et al. 2014).
- Hemogram analysis; neutropenia or neutrophilia, as well as the presence of a left shift, have been reported as having no prognostic value in septic cats (DeClue 2011; Klainbart et al. 2017), although the authors recommend further investigation as other studies have shown opposite results (Nivy et al. 2013). Toxic neutrophil changes, however, have been associated with poorer outcomes (Klainbart et al. 2017). A recent study evaluated the neutrophil-to-lymphocyte ratio in septic cats and found this a good measure to be used as a prognostic factor (Gori et al. 2021).
- Blood glucose; concerning glycemia as a prognostic factor for mortality in septic cats, many studies have failed to show an association (Costello et al. 2004; Ray et al. 2009; DeClue et al. 2011), but one report established that hyperglycemia upon admission is associated with increased mortality rates in septic cats, as reported for humans and dogs (Capes et al. 2000; Ray et al. 2009; DeClue et al. 2011; Scotti et al. 2019). It has been suggested that this might be due to decreased glycemic control in cats and decreased conversion of glucose to glycogen caused by decreased hepatic glucokinase activity (Van Vught et al. 2016).
- Serum albumin; hypoalbuminemia is a common finding in cats with SIRS and sepsis, due to decrease in hepatic production or increased vascular permeability and leakage (Brady et al. 2000; Costello 2015; Klainbart et al. 2017). However, no association with outcome has been made in both cats and dogs (King 1994; DeClue 2011). In human septic patients, low serum albumin values have been reported as a negative prognostic factor (Arnau-Barrés et al. 2019; Cakir and Turan 2021; Hu et al. 2021) as well as albumin changes during the first three days at ICU (Takegawa et al. 2019). Some studies, nonetheless, report an even more significant association between outcome and lactate-albumin ratios or neutrophil-albumin ratios (Chebl et al. 2020; Gharipour et al. 2020; Gong et al. 2020; Cakir and Turan 2021).
- Alanine Transaminase (ALT); increased ALT serum values have been correlated with the outcome in septic dogs (Winkler and Greenfield 2000). In cats, reports of medium serum ALT activity were higher in non-survivors, although a direct association with outcome was not made (Costello et al. 2004; DeClue 2011).
- Serum chloride; hypochloridemia has been associated with poorer prognosis in cats with sepsis (DeClue 2011). Conversely, one other study showed no association with outcome (Klainbart et al. 2017).
- Ionized calcium; one study evaluated the prognostic value of ionized calcium values in septic cats' outcomes. The occurrence of hypocalcemia upon admission was not associated with a

negative outcome; however, patients who did not normalize their serum calcium values during hospitalization were less likely to survive (Kellett-Gregory et al. 2010). Additionally, triglycerides have not been associated with death in these patients (Klainbart et al. 2017).

- Lactate levels; although lactate values are now incorporated in the current definition of septic shock in human patients, some studies have failed to report prognostic value in cats with sepsis (Scotti et al. 2019). However, other studies have conversely shown this association in cats, dogs and human patients (Chan et al. 2006; Kellett-Gregory et al. 2010; Liu et al. 2019; Gharipour et al. 2020). In dogs, hyperlactatemia and the inability to normalize lactate values during hospitalization is associated with a negative outcome (Chan et al. 2006).
- Coagulation tests; only one study has evaluated hemostatic findings in cats with sepsis, and concluded that PT, aPTT and platelet count were not associated with the outcome, although values were increased in the sepsis group (Klainbart et al. 2017). This differs from human studies that have reported prognostic value for aPTT, PT and antithrombin (Aird 2003). In dogs, lower antithrombin values have also been associated with poorer outcomes (Laforcade et al. 2008; Bentley et al. 2013).
- Disseminated Intravascular Coagulation (DIC); In regards to DIC, Klainbart et al. (2017) reported no association with outcome in cats, although this result was attributed to the small sample size. In humans, however, this association has been made, with positive DIC cases indicating a worse prognosis (Iskander et al. 2013).
- Total thyroxine (tT4); low serum tT4 concentrations have shown to be statistically associated with a negative outcome in cats with FPV infection (Petini et al. 2020).

In addition, several clinical interventions can be critical for the survival of severely ill cats with sepsis, including:

- Antibiotic treatment; administration of appropriate empirical antibiotics upon admission has been associated with positive outcomes in cats, dogs and humans (Kumar et al. 2006; Arnau-Barrés et al. 2019; Scotti et al. 2019). The hour-1 bundle most recently added to the Surviving Sepsis Campaign (SSC) guidelines recommends administration of empiric antibiotics almost immediately after presentation, as it has been shown to increase survival rates in humans (Levy et al. 2018; Evans et al. 2021).
- Fluid resuscitation; another SCC recommendation is the use of crystalloids for fluid resuscitation, although caution must be taken as a positive fluid balance after stabilization has been associated with worse outcomes both in cats and human patients (Acheampong and Vincent 2015; Troìa et al. 2019).
- Blood product transfusions; lastly, fresh frozen plasma (FFP) transfusions have been reported to be unfavorable and to decrease survival rates in humans, although no studies evaluating FFP transfusions have been done in septic dogs and cats (Qin et al. 2021).

2.1.5. Clinical Presentation and Diagnosis in Cats

2.1.5.1. Clinical Presentation in Cats

The clinical manifestations of sepsis are linked to the systemic inflammatory response, the infection and the organ dysfunction which results from the aforementioned factors. According to Brent (2017) and Angus and van der Poll (2013), we can differentiate some of the clinical manifestations of the systemic inflammatory response from infection foci and organ dysfunction, although an overlap between them may occur. Signs of systemic inflammatory response include fever, hypothermia, rigors, lethargy, hypo and anorexia, tachycardia and tachypnea with respiratory distress, altered mental status, hypotension, among others (Brent 2017; Cecconi et al. 2018).

In septic patients, an initial hyperdynamic phase is followed by a hypodynamic phase, during the evolution of the ongoing inflammatory process (Brady et al. 2000; Costello 2015). The hyperdynamic phase is characterized by presence of a fever, tachycardia and hyperemic mucous membranes owing to peripheral vasodilation. The following hypodynamic phase is its compensatory reaction, with tachycardia, vasoconstriction, pale mucous membranes, prolonged capillary refill time (CRT) and poor to absent pulse. This has been described mainly in human patients and dogs (Costello 2015). In contrast, it has been postulated that cats lack or have a shorter initial hyperdynamic phase, exhibiting solely the late hypodynamic phase, and developing bradycardia instead of tachycardia (Brady et al. 2000; Greiner et al. 2008; Costello 2015; Scotti et al. 2019). This phenomenon is not fully understood, although it was proposed by Schwartz et al. (1973) that the mechanism behind the onset of bradycardia might be related to simultaneous stimulation of vagal and sympathetic baroreceptors in response to hypotension.

2.1.5.1.1. Clinical Findings

One of the most common, though unspecific, findings in cats with sepsis is anorexia or hyporexia (Parsons et al. 2009; Forman 2017; Klainbart et al. 2017). Upon physical examination, septic cats are usually lethargic and depressed, and present pale mucous membranes or jaundice (Brady et al. 2000; Parsons et al. 2009). Cats may have weak or non-palpable pulses due to a severe hypovolemic state (Brady et al. 2000). Most cats demonstrate abdominal pain upon palpation, even when the infection foci is elsewhere (Brady et al. 2000; Costello et al. 2004; Parsons et al. 2009), and vomiting has been reported in 20-48% of septic cats (Parsons et al. 2009; Klainbart et al. 2017).

Most cats with sepsis experience bradycardia and hypothermia, although these are not directly associated (Brady et al. 2000; Costello et al. 2004; Greiner et al. 2008; Parsons et al. 2009; Costello 2015; Klainbart et al. 2017; Hiebert et al. 2022). On the other hand, some

studies report some septic cats with ongoing fever (Greiner et al. 2008; Parsons et al. 2009; Klainbart et al. 2017).

Tachypnea is a common finding in septic cats, including those with no evidence of primary pulmonary or thoracic disease (Brady et al. 2000; Costello et al. 2004). As described further on, the proinflammatory and procoagulant states lead to pulmonary hypertension, capillary protein leakage with development of pulmonary edema, microthrombi and type II pneumocyte hyperplasia, among other lesions, all of which lead to pulmonary dysfunction and, therefore, tachypnea and respiratory distress (Cardinal-Fernandez et al. 2017; Jarczak et al. 2021).

Some other clinical signs such as peripheral edema or ascites may occur as a consequence of hypoalbuminemia, hypotension and increased vascular permeability (King 1994; Parsons et al. 2009; Montealegre and Lyons 2021). However, a study concerning septic cats found that none of the cats which had low serum albumin values had developed peripheral edema (Costello et al. 2004).

In neonatal sepsis in kittens, the reported clinical signs are weakness, hypothermia, diarrhea, respiratory abnormalities, hematuria, cyanosis and eventually sloughing of parts of their extremities. In some cases, death can occur superacutely before evidence of any clinical signs (Hoskins 1993).

2.1.5.1.2. Clinical Laboratory Findings

Regarding erythrogram, common findings are nonregenerative anemia (Hoskins 1993; Brady et al. 2000; Costello et al. 2004; Klainbart et al. 2017), and metarubricytosis (Klainbart et al. 2017). There are many potential causes for anemia, and it is most probably multifactorial (Brady et al. 2000; Aird 2003; Costello et al. 2004). Increased activation of macrophages and neutrophils may potentiate premature erythrocyte clearance by the reticuloendothelial system, leading to low-grade hemolysis (Weiss and McClay 1988; Brady et al. 2000; Aird 2003; Costello et al. 2004). Additionally, cats' erythrocytes have increased susceptibility to oxidative damage, resulting in Heinz body production (Christopher et al. 1990; Brady et al. 2000; Costello et al. 2004). In inflammatory states, erythropoiesis can be flawed and the decreased production of erythrocytes can also explain the sepsis-induced anemia (Brady et al. 2000; Aird 2003; Costello et al. 2004). Lastly, blood loss from gastrointestinal ulceration or perforation can aggravate the condition (Aird 2003; Costello et al. 2004).

Concerning leucogram, leukocytosis is the most common finding, although it is included as one of the SIRS defining criteria (Brady et al. 2000; Aird 2003). Neutrophilia with a left shift is observed, with incidence in cats ranging from 60 to 73% (Hoskins 1993; Brady et al. 2000; Costello et al. 2004; Greiner et al. 2008; Klainbart et al. 2017). Many mechanisms contribute to this finding, such as increased neutrophil production and release from the bone marrow, as well as neutrophil demargination (Aird 2003). Toxic neutrophils may be observed and are

associated with left shift (Costello et al. 2004; Greiner et al. 2008; Klainbart et al. 2017). In some cases, leukopenia by neutropenia occurs due to bone marrow exhaustion, arrested development of granulocytic cells or imbalance between production and release of these cells into the bloodstream (Shoup et al. 1998; Aird 2003). Cats with sepsis demonstrate lymphopenia, monocytosis and eosinopenia, although monocytosis appears to be more common in septic dogs (Greiner et al. 2008; Klainbart et al. 2017).

Hyponatremia and hypochloridemia are both electrolyte disturbances characteristic of SIRS and sepsis in cats (Brady et al. 2000; DeClue et al. 2011; Klainbart et al. 2017). These alterations are a probable result of gastrointestinal, renal or third-space loss, depending on the infectious cause (Morais and Biondo 2012). In panleukopenia virus infection cases, gastrointestinal losses due to gut epithelial lesions would describe the low values of both sodium and chloride (Klainbart et al. 2017). One other electrolyte finding is hyperkalemia, which may be due to metabolic acidosis or as a consequence of renal injury (Brady et al. 2000; DeClue 2011).

Both hypoproteinemia and hypoalbuminemia are very common findings in cats diagnosed with sepsis and SIRS (King 1994; Brady et al. 2000; Costello et al. 2004; Parsons et al. 2009; DeClue 2011; Klainbart et al. 2017; Montealegre and Lyons 2021). It has been suggested that hypoalbuminemia might be due to increased capillary loss explained by increased permeability and leakage that characterize sepsis-induced vasodilation and hypotension, as well as a decrease in protein production in the liver attributed to both hepatic dysfunction and the acute phase response which takes place (Brady et al. 2000; Costello et al. 2004; Montealegre and Lyons 2021). Other causes include effusion loss, malnutrition due to anorexia and dilutional effects promoted by activation of antidiuretic hormone (ADH) (King 1994; Klainbart et al. 2017). In one study comparing septic cats and cats with non-infectious SIRS, it was shown that the former demonstrated a more severe hypoalbuminemia than non-infectious SIRS cats (DeClue 2011).

Regarding glycemia, most cats with sepsis have been reported as hypoglycemic (Hoskins 1993; Brady et al. 2000; Costello 2015), although many studies mention that this glycemic response is heterogeneous, as some patients demonstrate a hypoglycemic response while others exhibit hyperglycemia, or even an initial transient hyperglycemia and posterior glycemic decline (Postel and Schloerb 1977; King 1994; Costello et al. 2004; Greiner et al. 2008; Parsons et al. 2009; Costello 2015). It has been suggested that blood glucose concentration changes might occur due to increased gluconeogenesis, resulting in initial hyperglycemia and posterior hypoglycemia (Frezoulis et al. 2022).

Hyperbilirubinemia and icterus have been described in many septic cats and may be caused by liver and gallbladder-related issues as well as hemolytic anemia (Brady et al. 2000; Costello et al. 2004; Sergeeff et al. 2004; Klainbart et al. 2017; Troìa et al. 2019). Nonetheless,

most studies demonstrate normal or increased alkaline phosphatase (ALP) values (King 1994; Brady et al. 2000; Costello et al. 2004; Greiner et al. 2008; Hiebert et al. 2022). Serum γ-glutamyltransferase (GGT) activity has been reported high in approximately 63% of septic cats (Costello et al. 2004).

Hypertriglyceridemia is a common finding in septic cats (Cetinkaya et al. 2014; Klainbart et al. 2017). Some authors suggest that this might occur due to an increase in hepatic secretion of very-low-density lipoprotein (VLDL), a decrease in VLDL and triglyceride removal or both (Cetinkaya et al. 2014).

Increased creatine kinase (CK) and aspartate aminotransferase (AST) were reported in cats with sepsis (Klainbart et al. 2017). This might be due to a rhabdomyolysis-like status observed in some cats, where hypotension, shock, and muscle hypoxia and ischemia promote muscle damage. In other cases where a direct muscle injury takes place, the increase in CK and AST might be due to the infection site (Klainbart et al. 2017).

Serum ionized calcium concentrations can be decreased in septic patients, with incidences of 59 to 89% in cats (Costello et al. 2004; Kellett-Gregory et al. 2010; DeClue 2011). The underlying causes of hypocalcemia are not well understood, although some authors suggest parathyroid suppression, parathyroid hormone and vitamin D resistance or inadequate serum vitamin D concentrations (Zaloga et al. 1987).

The hemostatic abnormalities characteristic of SIRS and sepsis can take on a hypercoagulable form at first, with a following hypocoagulable state as the disease progresses. In most cases, DIC diagnosis only takes place in later stages, as the hypercoagulable phase is of difficult diagnosis and often clinically silent (Silverstein and Otto 2023). Nonetheless, DIC is common in dogs and cats with sepsis (Norris et al. 1999; Laforcade et al. 2003; Silverstein and Otto 2023). In one study, only 18% of cats with sepsis developed DIC, and any signs suggesting thrombosis and clinical bleeding were not evident (Klainbart et al. 2017).

Cats with sepsis have longer aPTT and PT than healthy cats, demonstrating an imbalance in the coagulation cascade with clotting factor consumption, although initially these markers can be within the normal range (Klainbart et al. 2017; Silverstein and Otto 2023). D-dimer plasma concentrations were reported as increased in cats with sepsis, indicating an increase in fibrinolysis (Klainbart et al. 2017). Furthermore, absolute thrombocytopenia is another common finding, with reports suggesting an incidence of approximately 35% (Hoskins 1993; Aird 2003; Klainbart et al. 2017; Silverstein and Otto 2023). In septic patients, protein C and antithrombin activities are usually reported as low (Klainbart et al. 2017), and protein C depletion is thought to be owing to increased consumption, decreased synthesis, neutrophil elastase degradation and vitamin K deficiency (Hopper and Bateman 2005). Being an anti-inflammatory mediator, its depletion may aggravate inflammation.

2.1.5.2. SIRS and Sepsis Diagnosis in Cats

As mentioned previously, the SIRS criteria continue to be used in the diagnosis of sepsis in dogs and cats (Montealegre and Lyons 2021). The gold standard for sepsis confirmation is blood culture, although cytology, histopathology and serology can also be performed (DeClue 2017; Ershad et al. 2019; Silverstein and Otto 2023). It should be noted that sepsis infection can be caused by other pathogens other than bacteria, although in most cats the main isolated organisms are Gram-negative bacteria (Costello et al. 2004). In some cases, blood culture might not be possible or viable and a presumptive diagnosis may be necessary, based on the clinical condition (DeClue 2017). In FPV infections, onset of sepsis is due to bacterial translocation from the gut to the bloodstream, caused by viral lesion of the intestinal mucosal barrier, combined with FPV-induced leukopenia, promoting an infection in an immunocompromised patient (Krentz and Allen 2017).

2.1.6. Multiple Organ Dysfunction Syndrome

Multiple Organ Dysfunction Syndrome (MODS) is characterized as dysfunction of two or more organ systems in critically ill patients such that intervention is needed to maintain homeostasis (Bone et al. 1992). It is most associated to sepsis and septic shock, although it may occur due to non-infectious conditions, such as trauma, neoplasia or SIRS. As mentioned previously, many mechanisms during SIRS and sepsis contribute to the induction of organ failure, such as systemic inflammation, cellular hypoxia, mitochondrial dysfunction and tissue damage (Osterbur et al. 2014). Many different forms of organ dysfunction have been identified in animals and human patients, although emphasis will be made to the predominantly clinically characterized ones.

2.1.6.1. Cardiac Dysfunction in Cats

Hypoperfusion may also occur due to cardiovascular dysfunction, which has been reported in septic humans, dogs and cats (Parker et al. 1984; Natanson, Danner, et al. 1989; Babyak and Sharp 2016). Epidemiology data concerning sepsis-induced cardiovascular dysfunction in cats, however, is lacking, although one study noted a 42% incidence of cardiovascular dysfunction in this population, which was associated with worse outcomes (Troìa et al. 2019).

Sepsis-induced cardiovascular dysfunction is thought to be caused by depression of the cardiac myocytes and mitochondrial dysfunction, both of which are caused by TNF-α and IL-1β (Natanson, Eichenholz, et al. 1989; Varani and Ward 1994; Kumar et al. 1996). Sepsis-induced cardiomyopathy is characterized by left ventricle (LV) intrinsic contractility depression, with systolic and diastolic dysfunction associated with low LV filling pressures, as well as decrease in right ventricle ejection fraction (Parker et al. 1984; Natanson, Danner, et al. 1989; Jones and Puskarich 2009; Vieillard-Baron 2011; Hotchkiss et al. 2016).

Regarding cats, an increased left ventricular end-diastolic pressure and decreased contractility of the LV has been observed in experimentally induced endotoxemia (Solis and Downing 1966; Hinshaw 1974). This finding should explain the onset of bradycardia in these cats, as a result of reflex vagal stimulation due to stretch of LV receptors (Oberg and Thorén 1973; Thorén 1973).

2.1.6.2. Acute Respiratory Distress Syndrome in Sepsis (ARDS)

The lungs are primarily affected in MODS and respiratory dysfunction has been reported in 26 out of 43 septic cats (Troìa et al. 2019).

Alterations to the alveolar-endothelium barrier in the lungs caused by many uncontrolled proinflammatory cytokines and mediators promote protein-rich fluid accumulation in the interstitium and alveoli (Tomashefski 2000; Pelosi et al. 2003; Hotchkiss et al. 2016; Cardinal-Fernandez et al. 2017; Huppert et al. 2019; Matthay et al. 2019). Simultaneously, type II pneumocyte lesion decreases surfactant production and the existing surfactant is inactivated by fluid plasma proteins, resulting in increasing interalveolar surface tension and, consequently, diffuse microatelectasis (Cardinal-Fernandez et al. 2017). All the described mechanisms cause decreased ventilation, hypoxia and a decrease in lung compliance, leading to acute respiratory distress syndrome (ARDS) (Hotchkiss et al. 2016; Cardinal-Fernandez et al. 2017; Jarczak et al. 2021). ARDS may lead to further organ dysfunction, such as liver, kidney, cardiovascular and central nervous system dysfunctions (Jarczak et al. 2021).

2.1.6.3. Sepsis-induced Acute Kidney Injury

The pathophysiology behind sepsis-associated acute kidney injury (sa-AKI) is not yet fully understood, due to lacking histopathologic investigations (Langenberg et al. 2008). It is suggested that the coagulation issues that cause microvascular occlusion can lead to a diminished blood flow to the kidneys and, consequently, necrosis of the tubular epithelial cells and tissue damage (Schrier and Wang 2004; Verma and Molitoris 2015). As such, one of the probable causes of AKI in these patients might be hypovolemia and shock (Uchino et al. 2005). Some authors suggest ischemic injury caused by proinflammatory cytokines and reactive oxygen species (Verma and Molitoris 2015; Jarczak et al. 2021). Furthermore, the vasodilation and vascular leakage described earlier, when occurring in the renal tissues, lead to edematous peritubular distension, resulting in decreased oxidative phosphorylation. These mechanisms lead to blood flow redistribution in the kidney and renal medulla hypoperfusion (Jarczak et al. 2021).

The prevalence of sa-AKI in animals is unknown. However, some studies report an incidence of 14% in septic dogs and, in a different study, renal dysfunction was present in 18 out of 43 septic cats (Kenney et al. 2010; Troìa et al. 2019)

2.1.6.4. Hepatobiliary Dysfunction

Hepatic dysfunction in MODS context is determined when presence of hyperbilirubinemia takes place in cases where there was no existing liver dysfunction (Singer et al. 2016). Liver hypoperfusion in septic shock leads to diminished protein synthesis and lactate clearance, glycogenolysis and gluconeogenesis with subsequent hypoglycemia (Maynard et al. 1997; Szabo et al. 2002; Aronsohn and Jensen 2011; Gyawali et al. 2019). The acute cellular and mitochondrial injury which consequently take place causes enzyme leakage and an elevated serum ALT activity. In a second instance, secondary hepatic dysfunction occurs via interaction of Kupffer cells, hepatocytes, neutrophils and endothelial cells in response to systemic inflammation (Szabo et al. 2002; Aronsohn and Jensen 2011). The proinflammatory mediators also damage cholangiocyte barrier functions and cholestasis takes place (Szabo et al. 2002; Aronsohn and Jensen 2011; Gyawali et al. 2019). Although sepsis-induced cholestasis has been described in dogs, its histopathologic characteristics have not been reported in cats (Taboada and Meyer 1989; King 1994; Brady et al. 2000; Kenney et al. 2010). However, hepatic function is known to be mainly affected in septic cats (Babyak and Sharp 2016; Troìa et al. 2019).

2.1.6.5. Critical Illness-Related Corticoid Insufficiency

Critical illness-related corticoid insufficiency (CIRCI), formerly known as relative adrenal insufficiency, is a condition where the body fails to produce enough cortisol to meet the heightened demand during critical illness (Annane 2008; DeClue 2014a; Silverstein and Otto 2023). Diagnosis is usually determined by an inadequate response to exogenous ACTH administration, although this is controversial and current recommendations do not support the ACTH stimulation test for these patients (Pisano et al. 2017; Evans et al. 2021; Silverstein and Otto 2023). Currently, CIRCI is suspected if the patient with refractory hypotension after fluid and vasopressor therapy responds to administration of hydrocortisone (Singer et al. 2016; Evans et al. 2021). The cause of CIRCI in septic patients has been suggested to be a multifactorial phenomenon, where dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis, altered glucocorticoid metabolism pathways, tissue resistance to glucocorticoids and decreased receptor sensitivity may all play roles (Annane 2008; Kanczkowski et al. 2015; Silverstein and Otto 2023). Although reports of CIRCI in veterinary literature are rare, it has been reported in both dogs and cats and a recent study demonstrated successful treatment in a septic cat with CIRCI (Burkitt et al. 2007; Pisano et al. 2017).

2.1.6.6. Neurological Dysfunction

Septic patients usually present with an abnormal mentation status, most probably due to sepsis-associated encephalopathy (SAE) (Osterbur et al. 2014; Gyawali et al. 2019). This is an acute deterioration of mental status which is characterized by behavioral changes, as

well as decreased consciousness, awareness and cognition. Although SAE has been reported in human septic patients, the incidence in animals is unknown (Osterbur et al. 2014). Altered mentation in veterinary patients is manifested by lethargy, decreased response to external stimuli, tendency to stay in one location and drink or eat less. In more aggravated cases, stuporous animals may remain in sternal or lateral recumbency and only react to noxious stimuli. Comatose animals may present similar clinical signs as stuporous animals but do not react to any stimuli whatsoever (Bagley and Platt 2014).

The pathophysiology of SAE in human patients is not well understood, although theories suggest that it may be multifactorial, including a decrease in blood flow to the brain, glutamate excitotoxicity and crossing of the cytokines through the blood-brain barrier (Iskander et al. 2013; Osterbur et al. 2014). Additionally, the stimulation of the endothelial cells may promote microcirculatory dysfunction and coagulation defects, along with a decrease in vascular tone, resulting in hemorrhaging and ischemic lesions (Osterbur et al. 2014).

This central nervous system dysfunction has been reported to be associated with a poorer prognosis in human patients and may cause long-term injurious consequences in survivors (Sprung et al. 1990). The long-term impact in animal patients is unknown (Osterbur et al. 2014).

2.1.7. Treatment

Current recommendations for sepsis treatment can be divided in two sections: causal therapy and supportive therapy (Jarczak et al. 2021). Causal therapy includes interventions to eliminate the main infection focus such as surgery and antibiotics (Hotchkiss et al. 2016). On the other hand, supportive therapy has a more individually tailored approach, where the patients' unique response is observed and certain interventions are made in order to attenuate the organ damage and maintain stability (Jarczak et al. 2021). Some examples are vasopressor administration, mechanical ventilation or renal replacement therapy (Jarczak et al. 2021).

The Surviving Sepsis Campaign (SSC) was created in 2004 for sepsis treatment protocols to be implemented and comprehended, in order to improve survival in these patients. In this campaign, "sepsis bundles" were defined as certain measures to be taken in a specific period of time and have been reported as successful in septic patient treatment (Levy et al. 2018; Evans et al. 2021). This concept derived from the Early Goal Directed Therapy (EGDT) human trial, which addressed resuscitation of septic and septic shock patients (Rivers et al. 2001). This trial determined that in sepsis, the key to a successful recovery is an early start of interventions, a statement which has been widely accepted (Jarczak et al. 2021). In the last SSC (2021), the "Hour-1 bundle" was introduced, replacing the 3 and 6-h bundles previously implemented (Levy et al. 2018; Evans et al. 2021). The "Hour-1 bundle" encompasses 5 main interventions: blood cultures prior to antibiotic administration, initiation of broad-spectrum

antibiotics, administration of intravenous fluids, vasopressor application when hypotensive and serum lactate measurement. Although more than 1 hour may be necessary for complete resuscitation, this bundle implicates the initiation of resuscitation almost immediately, during the first hour after sepsis onset (Levy et al. 2018; Evans et al. 2021).

2.1.7.1. Causal Therapy

2.1.7.1.1. Antibiotics and Source Control

The most recent SSC (2021) guidelines recommend administration of antimicrobials immediately (Ferrer et al. 2014; Levy et al. 2018; Mi 2019; Evans et al. 2021). According to Levy et al. (2018), blood cultures and antibiograms should be performed, although without delaying antimicrobial therapy. Many studies have demonstrated increased mortality when source control and antibiotics were delayed (DeClue 2014b; Kumar 2014; Hotchkiss et al. 2016; Whiles et al. 2017) and others showed that empirical antibiotics in the first hour of sepsis or septic shock onset in human patients reduced mortality (Kumar et al. 2006; Ferrer et al. 2014; Sankar et al. 2021). This topic is controversial, as many authors disagree with these guidelines, and mention that onset of immediate antibiotics may lead to overdiagnosis and potentiate antimicrobial resistance (Kalil et al. 2018; Freund et al. 2019). Nonetheless, initiation of broad-spectrum intravenous empirical antibiotics with prior blood draw and culture within the first hour after sepsis or septic shock recognition is recommended. When culture results are available, antimicrobial doses should be adjusted and, if necessary, the chosen antibiotic should be changed to a narrower option which covers the causal agent (Wheeler and Bernard 1999; Hotchkiss et al. 2016; Cecconi et al. 2018; Levy et al. 2018; Jarczak et al. 2021). The dose correction of the antibiotics should take consideration the pharmacokinetic alterations that occur in these patients, as each organ's dysfunction may interfere with the drug metabolism, distribution and clearance (Jarczak et al. 2021).

As infection identification might not be possible, administration of antibiotics should be considered as soon as possible to ensure pathogen clearance (DeClue 2014b). The choice of empiric antibiotic is ultimately an individual and patient-specific decision, depending on the infection foci, the probable pathogens and taking into consideration local susceptibility patterns (Brent 2017; Gyawali et al. 2019). Suggestions of combined antibiotics which cover Gram-negative and -positive aerobes and anaerobes are: ampicillin or clindamycin with enrofloxacin, amikacin, gentamicin or ceftazidime (Kim et al. 2020; Odabasi and Bulbul 2020; Silverstein and Otto 2023).

Lastly, source control via surgery or any intervention that can remove the infection focus can be necessary, especially in bite wounds, fractures or penetrating trauma cases (Costello 2015; Brent 2017; Gyawali et al. 2019).

2.1.7.2. Supportive Therapy

2.1.7.2.1. Fluid Therapy

As mentioned earlier, SIRS and sepsis lead to hypovolemia due to fluid extravasation, a consequence of vasodilation and endothelial damage characteristic of the onset of inflammation in these patients. This ultimately leads to hypoperfusion, hypoxia and, consequently, organ dysfunction (Byers 2017). As such, initial hemodynamic resuscitation is considered a major cornerstone in the initial therapeutic approach of human and animal septic patients, although no guidelines have been made for dogs and cats (DeClue 2017; Levy et al. 2018; Evans et al. 2021; Montealegre and Lyons 2021). Current guidelines for human sepsis recommend 30 mL/Kg of crystalloid intravenously during the first hour of resuscitation (Levy et al. 2018; Evans et al. 2021). However, given the species-specific differences in fluid tolerance and blood volume, as well as the different clinical presentations and diversified degrees of hypovolemia in dogs and cats, caution must be taken and some authors suggest an individual and tailored approach to each patient (Montealegre and Lyons 2021). Incremental isotonic crystalloid fluid boluses of 10-15 ml/Kg can be implemented in hypoperfused patients, provided that constant reassessment is assured to determine whether they are fluid responsive (Montealegre and Lyons 2021). Following resuscitation, fluid therapy might still be needed to maintain euhydration (Montealegre and Lyons 2021).

Considering the type of fluid, Ringer's Lactate (LR) has shown favorable results in comparison to 0.9% sodium chloride (NaCl), with the former being associated with a lower mortality rate in these patients. Chloride-rich solutions are known to decrease renal perfusion and induce AKI in humans and dogs, as well as to promote inflammatory shift, induce shock in animal models and metabolic acidemia (Madhusudan et al. 2014). Synthetic colloids such as hydroxyethyl starch (HES) in addition to crystalloids could be used to prevent edema and fluid loss, although it is controversial in septic patients, as it has been reported as inducing renal injury in human patients (Brunkhorst et al. 2008; Mutter et al. 2013; DeClue 2017). Some studies have evaluated its effect on cats and did not observe association with kidney injury or increased mortality (Sigrist et al. 2017; Yozova et al. 2017). However, as there might be potential risk of renal injury and given that there is no evidence of benefits in these patients, it is no longer recommended (Montealegre and Lyons 2021).

2.1.7.2.2. Vasopressors

If the patient is unresponsive to fluid resuscitation, vasopressors may be needed to increase blood pressure (DeClue 2017; Evans et al. 2021). Early use of vasopressors is associated with positive outcomes in human sepsis patients, and therefore is recommended in septic shock cases. Norepinephrine with 0.1-1 mcg/Kg/min doses is recommended, promoting vasoconstriction and an increase in mean arterial pressure (Hamzaoui et al. 2010;

Evans et al. 2021; Jarczak et al. 2021; Montealegre and Lyons 2021). Some studies suggest that it might improve microcirculatory flow additionally, with increases in global oxygen delivery and improving tissue oxygenation (Jhanji et al. 2009). According to the SSC guidelines, if mean arterial pressures are still inadequate after norepinephrine administration, addition of vasopressin is suggested instead of increasing norepinephrine doses (Evans et al. 2021). When the patient is in septic shock and has evidence of systolic cardiac dysfunction, dobutamine can be added (5-15 mcg/Kg/min) to norepinephrine (Montealegre and Lyons 2021; Silverstein and Otto 2023). Although vasoconstricting drugs increase arterial blood pressure, promoting normal blood flow and oxygen delivery to the body, caution must be taken as they can also cause renal or splanchnic vasoconstriction and cause the opposite effect, leading to organ ischemia and dysfunction (Silverstein and Otto 2023).

2.1.7.2.3. Transfusions

It is not yet clear whether albumin transfusions improve outcomes in septic patients. One human study reported no difference in hospital length of stay, organ failure and mortality in ICU patients, but when individualizing septic patients, albumin transfusions were associated with a better outcome (Finfer et al. 2004). Other studies have demonstrated positive outcomes associated with this treatment, suggesting that some patients may benefit from albumin transfusions (Delaney et al. 2011). Studies in animals are lacking, however, and there are no current studies in cats. One study in septic dogs found no association between albumin transfusion and a positive outcome (Horowitz et al. 2015).

The use of blood products for transfusions depends on the individual patient's status. Packed red blood cell (RBC) or fresh whole blood transfusions may be indicated in cases where the animal is anemic with extremely low hematocrit values or when thrombocytopenia is present (Gyawali et al. 2019; Silverstein and Otto 2023).

Fresh frozen plasma (FFP) transfusions, however, are only indicated when there is presence of concurrent coagulopathy with bleeding evidence, although it had been reported to decrease TNF-α and syndencan-1 levels in critically ill patients (Straat et al. 2015; Montealegre and Lyons 2021; Qin et al. 2021). This suggests a potential role in glycocalyx and endothelial maintenance as well as decrease of inflammation, and some authors suggest that plasma transfusions may be superior in maintaining the glycocalyx compared to crystalloids (Straat et al. 2015; van den Brink et al. 2021; Montealegre and Lyons 2021). Nonetheless, further studies are needed as clinical implications of these transfusions are unknown, and some studies have failed to show improved outcomes in septic patients receiving FFP (van den Brink et al. 2021; Montealegre and Lyons 2021; Qin et al. 2021). On the other hand, FFP transfusions can be indicated in patients with severe hypoalbuminemia instead of albumin transfusions in order to

increase oncotic pressure and lessen fluid loss to the interstitium (Montealegre and Lyons 2021; Silverstein and Otto 2023).

2.1.7.2.4. Supportive care

Enteral or parenteral nutrition may help maintain barrier function and aid in recovery in an anorexic animal. Additionally, gastric protectants may also be administered, such as omeprazole, famotidine or sucralfate (Silverstein and Otto 2023).

Other possible supportive measures include oxygen supplementation in order to improve gas exchange and ensure optimal oxygen carrying capacity (DeClue 2017; Jarczak et al. 2021). In extreme cases, mechanical ventilation might be needed, as is described in human patients (DeClue 2017; Jarczak et al. 2021).

Lactate measurements are included in the most recent care bundle mentioned in SSC guidelines and should be performed promptly (Levy et al. 2018; Evans et al. 2021). Lactate is currently viewed as a good biomarker for SIRS and sepsis as it is considered a surrogate indicator of tissue hypoxia and hypoperfusion (Mikkelsen et al. 2009; Garcia-Alvarez et al. 2014; Levy et al. 2018). Increased serum lactate values serve as a good predictor of mortality in human patients, cats and dogs and a marker for disease evolution, as decreases in lactate values during hospitalization are associated with positive outcomes (Mikkelsen et al. 2009; Parsons et al. 2009; Cortellini et al. 2015; Innocenti et al. 2019; Liu et al. 2019; Jarczak et al. 2021). One study, however, failed to demonstrate association between normalization of lactate values during hospitalization and positive outcomes in cats (Scotti et al. 2019). Nonetheless, it is suggested to use this parameter to guide in hemodynamic management and fluid therapy volumes (Cecconi et al. 2018; Levy et al. 2018; Evans et al. 2021).

Finally, pain management should be considered as most septic patients exhibit signs of pain (Brady et al. 2000; Costello et al. 2004; Parsons et al. 2009). Analgesic administration with ketamine or buprenorphine may be an option (DeClue 2017).

2.1.7.3. Adjunctive Therapies

2.1.7.3.1. Glucocorticoids

The use of corticosteroids in sepsis treatment has been under debate for many decades. It was thought that the immunomodulatory effect they promote would attenuate the inherent inflammation that occurs, but many studies in human patients have demonstrated no positive outcomes whatsoever (Sprung et al. 2008; Costello 2015; Venkatesh et al. 2018). Nonetheless, current SCC guidelines recommend the use of hydrocortisone in patients with poor responses to fluid and vasopressor therapy and when onset of septic shock (Evans et al. 2021). Some authors suggest eventual administration of low doses of corticosteroids for relative adrenal insufficiency management (Silverstein and Otto 2023). There are reports of

CIRCI in both cats and dogs and one study showed successful CIRCI management with hydrocortisone in a cat with septic shock (Burkitt et al. 2007; Pisano et al. 2017).

2.1.7.3.2. Other Innovative Approaches

Although investigation concerning sepsis has evolved immensely in the past decades, no treatment has been proven to be effective. Extracorporeal blood purification therapies have been under investigation, as they remove both bacterial endotoxins and inflammatory mediators from the bloodstream but show no evidence of efficacy in septic patients (Honoré et al. 2012). Immunotherapy with antibodies against PD-1 and its corresponding receptor PD-L1 are promising candidates for sepsis treatment, and clinical trials have been performed (Hotchkiss et al. 2019). On the other hand, some studies show that IL-7 might be an option, and its efficacy in HIV patients has been demonstrated (Lévy et al. 2012). Polyvalent intravenous immunoglobulins (IVIG) may show effectiveness, as it positively affects anti- and pro-inflammatory processes. However, its use in septic patients is controversial as it has been shown to induce side effects, and SSC guidelines do not recommend the use of IVIG preparations with exclusively IgG for sepsis and septic shock (Evans et al. 2021). Clinical trials with nitric oxide (NO) inhibitors, statins, Toll-like receptor antagonists, HMGB1 inhibitors and NF-κB inhibitors have failed to show any positive results. Polymyxin E has been experimented on dogs with parvoviral enteritis and demonstrated positive benefits, although adverse effects occur commonly. Lastly, CD5L has shown promising results due to its pathogen-binding and anti-inflammatory properties in an experimental animal model of sepsis (Oliveira et al. 2022).

2.2. Feline Panleukopenia

2.2.1. Etiology and Epidemiology

Feline Panleukopenia (FP) or Feline Parvovirus Infection is considered as one of the most common infectious diseases in cats. It is mainly caused by Feline Panleukopenia Virus (FPV) but infection by related mink enteritis virus or canine parvovirus (CPV) may take place (Sykes and Parrish 2023). FPV causes enteritis and panleukopenia in wild and domestic cats, as well as other species such as minks and foxes. It does, additionally, have the ability to replicate itself in dogs' lymphoid tissues, without intestinal invasion and, therefore, without causing any clinical signs, illness or shedding (Sykes and Parrish 2023).

FPV is a small, non-enveloped, single stranded DNA virus, and its virulence is attributable to its stability in the environment, which makes it possible for indirect transmission to occur. FPV is characterized by having an incubation period of 2-7 days, a short plasma phase viremia of about 5 days and a short shedding period (1-2 days), with excretion of up to 10^9 viral particles per gram of feces during the acute intestinal phase of infection. Regardless, cases with shedding periods of up to 6 weeks post-infection have been reported (Sykes and Parrish 2023).

FP usually affects cats with 1 year or less but can occur in older unvaccinated or inadequately vaccinated cats. However, despite complete vaccination, maternally derived antibodies (MDA), which may be present until 16 weeks of age, may interfere with the vaccination (Sykes and Parrish 2023).

2.2.2. Pathogenesis and Clinical Findings

FPV replicates itself mainly in cells with high mitotic index, such as intestinal epithelium cells, lymphoid tissue cells, bone marrow and some cells that constitute the central nervous system (cerebellum, cerebrum, retina and optic nerves) (Sykes and Parrish 2023). As such, the gastrointestinal tissue suffers damage, with shortening of the villi and intestinal permeabilization, causing malabsorption. Moreover, its replication in lymphoid tissue cells results in lymphoid tissue necrosis and functional immunosuppression, with decreased T-cell activity. Leukopenia, which is characteristic of FP, develops due to viral replication in the bone marrow and neutrophil sequestration in the damaged gastrointestinal tract (Sykes and Parrish 2023).

Many factors contribute to disease severity, such as age, vaccination and immune status and concurrent infectious diseases. In adult cats, the most common presentation of the infection is subclinical (Sykes and Parrish 2023).

Clinical infection is characterized by fever, weakness, lethargy, and dehydration in a first instance. Vocalization, dehydration, vomiting, diarrhea (hemorrhagic or not), anorexia and weight loss may also occur. However, some cats show little to no gastrointestinal clinical signs, with presence of only anorexia and lethargy. Pain upon abdominal palpation or a hunched stance is frequent, suggesting abdominal pain. Oral ulceration is reported as consequence of high fevers and mucosal pallor may be present in severe cases. In rare instances, icterus may develop and in terminal cases cats become comatose, bradycardic and hypothermic. When neonatal infection takes place, kittens may demonstrate neurologic abnormalities such as ataxia, intention tremors, incoordination, hypermetria, and other defects, due to viral destruction of Purkinje cells which lead to cerebellar hypoplasia. These neurologic signs are nonprogressive and are more noticeable at 2-3 weeks of age, when kittens start walking. As other neurons may also be infected, ocular abnormalities can take place, such as optic nerve hypoplasia or retinal folding, dysplasia and degeneration (Sykes and Parrish 2023).

Gram-negative bacteria endotoxemia due to bacterial translocation to the bloodstream is a major complication in FPV infections, that can result in an activation of the innate immune response and possibly in the imbalance of the immune system that characterizes SIRS and, consequently, sepsis. Other complications of FP are DIC, which can be clinically identified by ecchymoses and petechial formations, as well as hemorrhaging. However, the absence of these does not discard the occurrence of DIC and at times the only demonstrative clinical sign is a slight thrombocytopenia. This complication can cause sudden death due to the

hypercoagulable state that potentiates microthrombi and, consequently, hypoxia and organ dysfunction (Silverstein and Otto 2023; Sykes and Parrish 2023).

2.2.3. Bacterial Translocation and Sepsis

The occurrence of SIRS and sepsis in cats with FP can be due to many factors. The disruptive effects of FPV on intestinal mucosa epithelial cells permit translocation of Gram-negative and anaerobic bacteria and endotoxins from the intestinal lumen into the bloodstream and onset of bacteremia and endotoxemia (Krentz and Allen 2017; Petini et al. 2020; Parrish and Sykes 2023). On the other hand, viral replication in the bone marrow and lymphoid tissues causes immunosuppression and leukopenia, which potentiates an inadequate host immune response to infection and allows for secondary infections to occur (Sykes and Parrish 2023). The onset of inflammation and organ dysfunction that can derive from this may lead to further aggravation of gastrointestinal barrier disruption by proinflammatory cytokines, perpetuating the cycle (Krentz and Allen 2017).

2.2.4. Diagnosis

2.2.4.1. Clinical Laboratory Findings

In FP, the most common complete blood count (CBC) finding is leukopenia, caused by both neutropenia and lymphopenia, and there may be toxic band neutrophils (Larsen et al. 1976; Kruse et al. 2010; Sykes 2013; Sykes and Parrish 2023). This hematological finding is caused by bone marrow suppression which occurs as a consequence of FPV replication in those tissues. However, absence of leukopenia does not exclude the differential diagnosis of FPV infection. Some cats may present leukocytosis, on the other hand, which may be due to a myeloid compensation response to the attack, with hyperplasia of the bone marrow (Sykes 2013; Sykes and Parrish 2023).

Thrombocytopenia is another common abnormality that occurs, most probably due to bone marrow lesion, megakaryocyte destruction or DIC (Kruse et al. 2010; Sykes 2013). Mild anemia is a common finding in cats with panleukopenia (Kruse et al. 2010; Sykes 2013; Sykes and Parrish 2023), although normal hematocrit, erythrocyte and hemoglobin values have been observed in some studies (Larsen et al. 1976). In cases where there is severe gastrointestinal blood loss, anemia may be marked. When mild anemia is present, it is usually classified as nonregenerative (Kruse et al. 2010).

Regarding serum biochemistry results, hypoalbuminemia is the most common finding in panleukopenic cats, and may result from albumin loss due to gastrointestinal leaking, low albumin production following hepatic lesion and from decreased protein intake (Sykes 2013; Sykes and Parrish 2023). Other common findings are electrolyte abnormalities, such as hypochloridemia, hyponatremia and hyperkalemia (Kruse et al. 2010; Sykes 2013; Porporato et al. 2018; Sykes and Parrish 2023). Hypokalemia, on the other hand, might occur less

commonly, and possible causes may be anorexia, vomiting or gastrointestinal losses (Kruse et al. 2010; Sykes and Parrish 2023). Hypoproteinemia is a relatively common finding and glycemia values vary, with 29% of cats developing hyperglycemia and a fewer 6% showing hypoglycemia, according to Kruse et al (2010). In severely affected cats, ALT, AST, creatinine and bilirubin serum values may be elevated (Sykes 2013; Sykes and Parrish 2023).

2.2.4.2. Diagnostic Testing

Polymerase chain reaction (PCR) assays are considered to be the gold standard of parvovirus infection diagnosis; however, this test can originate false positives in recently vaccinated cats (Patterson et al. 2007; Sykes 2013). Nonetheless, as these assays are usually quantitative PCR, depending on the laboratory in question it may be possible to determine the amount of viral particles and assume whether a positive result is due to natural infection, as opposed to vaccine interference. PCR assays detect parvovirus DNA in fecal or blood samples and, depending on the results, may help clinicians determine whether the patient is shedding and/or in viremia. This diagnostic test does not, however, differentiate CPV from FPV (Patterson et al. 2007; Neuerer et al. 2008; Sykes 2013).

Fecal antigen enzyme-linked immunosorbent assay (ELISA) test kits can be used for parvovirus diagnosis in a readily manner, both of CPV and FPV in fecal samples from dogs and cats. Nonetheless, false negatives may occur (Barrs 2019). Conversely, false positives may occur after vaccination with modified live vaccines (MLV) (Patterson et al. 2007; Sykes 2013). For this reason, caution should be taken in interpreting point-of-care tests and obtaining of a detailed patient history is always recommended.

2.2.4.3. Diagnostic Imaging

Abdominal radiography of cats with FP may show a gas and fluid-filled gastrointestinal tract, as well as poor serosal detail (Sykes 2013). Recent studies have analyzed gastrointestinal ultrasonographies of cats diagnosed with FP and determined that diffuse small intestinal mucosal layer thinning, muscular layer thickening and mucosal hyperechogenicity are some of the most common findings (Isaya et al. 2021). Some kittens develop neurological clinical signs and as such, magnet resonance imaging (MRI) or computerized tomography (CT) scan may reveal evidence of cerebellar hypoplasia or agenesis and, less commonly, hydrocephalus, hydrancephaly or porencephaly (Sykes 2013).

2.2.5. Treatment

2.2.5.1. Fluid Therapy and Blood Product Transfusions

Cats with panleukopenia can be severely dehydrated, a factor that highly contributes to mortality in these patients (Barrs 2019). Therefore, intravenous fluid therapy with crystalloids is mandatory in any hospitalized patient, and a regular monitorization of acid-base balance and electrolytes, with eventual electrolyte supplementation, is recommended. Blood glucose

should also be monitored regularly and corrected (Truyen et al. 2009; Sykes 2013; Barrs 2019; Sykes and Parrish 2023).

Many cats are hypoalbuminemic or hypoproteinic during hospitalization and, as such, are candidates for FFP transfusions or intravenous synthetic colloids in order to restore oncotic pressure. Whole-blood transfusions are also an option when patients are both anemic and hypoalbuminemic. Another option for anemic cats may be concentrated erythrocyte transfusion (Barrs 2019).

2.2.5.2. Antibiotics

Antimicrobial treatment is essential in patients with parvovirus infection, due to gut barrier lesion and bacteria translocation to the bloodstream. As such, treatment with parenteral broad-spectrum antibiotics, with efficacy against Gram-negative and anaerobic bacteria, is recommended (Parrish and Sykes 2023; Sykes and Parrish 2023). Some examples of the antibiotics that are used are amoxicillin with clavulanic acid or ampicillin, in combination with marbofloxacin or, if hydrated and with no evidence of acute kidney injury, gentamicin as a last resort (Barrs 2019; Parrish and Sykes 2023).

2.2.5.3. Antiemetics, Gastric Protectants and Feeding

Antiemetics should be administered when vomiting occurs and persists (Truyen et al. 2009). While vomiting persists, oral intake of water and food should be suspended although this is no longer recommended in dogs, and similar studies in cats should be performed. Nonetheless, early enteric feeding may be an option, as has been reported in dogs to accelerate recovery (Mohr et al. 2003). Enteric feeding via placement of nasoesophageal or esophagostomy tubes should be approached with caution in cats as aspirate pneumonias may occur, but there are no reports on enteral nutrition in cats to date (Sykes and Parrish 2023). Maropitant is usually the antiemetic of choice (Barrs 2019), but ondansetron may be effective (Sykes 2013; Barrs 2019; Sykes and Parrish 2023). Additionally, in intractable cases maropitant in combination with ondansetron can be used. In some cases where cats have gastric reflux and risk of developing esophagitis, gastric protectants such as pantoprazole or omeprazole may be administered parenterally (Barrs 2019).

2.2.5.4. Immunosuppressants

The use of Recombinant Feline Interferon omega (rFeINF) as treatment for parvovirosis in dogs has been investigated, resulting in decreased clinical signs and positive outcome (Martin et al. 2002). In contrast, studies which investigated the equivalent use in cats with risk of developing feline panleukopenia demonstrated no correlation with clinical signs or outcome, although serologic analyses showed a more reactive immune and less exacerbated acute inflammatory responses (Paltrinieri et al. 2007).

2.2.6. Prevention

2.2.6.1. Vaccination

Adequate vaccination should confer immunity and prevent FP in cats. It is thought that immunity can be present 1 week after vaccination of a single dose of an attenuated live viral vaccine and that this immunity may last a lifetime, as long as MDA no longer persist (Jas et al. 2009; Sykes and Parrish 2023). However, it is recommended that initial vaccination should consist of 2 or 3 doses of attenuated live viral vaccine, 3-4 weeks apart, from 8 weeks of age onward, with the last vaccine to be administered no earlier than 16-20 weeks of age (Truyen et al. 2009; Digangi et al. 2012; Jakel et al. 2012; Sykes and Parrish 2023). A booster should be given at 6 months to 1 year and, from then on, every 3 years (Day et al. 2016; Sykes and Parrish 2023). Caution should be taken in pregnant queens, as vaccination with attenuated live vaccines can cause cerebellar hypoplasia in the fetuses, or fetal loss (Sharp et al. 1999; Sykes and Parrish 2023).

2.2.6.2. Other Preventive Measures

When adopting new kittens, it is important to make sure that certain care is taken. If the household they are being introduced in has previously harbored FPV infected cats, cleaning and disinfection of the facilities with proper disinfectants (such as bleach) and a two-week waiting period is recommended (Barrs 2019; Sykes and Parrish 2023). Passive immunization with serum from cats with high antibody titers may be an option in outbreak circumstances, or when introducing into a shelter with unknown disease outbreaks status (Truyen et al. 2009; Sykes and Parrish 2023).

2.2.7. Prognosis

Although subclinical and mild infections are common, FPV infection can be devastating and survival rates range from 20 to 50% (Kruse et al. 2010; Porporato et al. 2018; Sykes and Parrish 2023). As explained earlier, there are many complications that might occur, such as DIC and sepsis, which ultimately decreases prognosis. Recovery in FPV infections takes longer than CPV infections in dogs; however, if a cat is to survive during the first 5 days of treatment, it is said to have a positive prognosis (Sykes and Parrish 2023).

3. Risk and Prognostic Factors for Systemic Inflammatory Response Syndrome in cats with Panleukopenia Virus Infection

3.1. Introduction and Objectives

Sepsis and SIRS are clinical entities associated with increased morbidity and mortality in both human patients and animals worldwide. Although sepsis and SIRS prevalence is not well documented in veterinary medicine, there are reports which show similar rates between cats and humans (Babyak and Sharp 2016). However, contrary to human studies, causes for

sepsis and SIRS in feline patients differ and these occur mainly due to septic peritonitis, urologic or gastrointestinal diseases. Sepsis and SIRS are considered to account for many deaths in the ICU, with reported mortality rates of up to 79% in cats. Although sepsis focused research has evolved and more information concerning sepsis' pathophysiology has come to light in recent years, studies in feline patients are lacking. This, in turn, enhances the urgent need for a broader and more comprehensive insight on feline SIRS in order to better identify, diagnose and manage in a timely manner. As such, the present study was conducted in order to tackle some of the current issues regarding SIRS identification and diagnosis in feline patients, as well as potentially identify prognostic factors, aiming for future effective treatment protocols and adequate management techniques.

Feline panleukopenia virus infection is an infectious disease which predisposes feline patients to sepsis and, as such, has been considered as a feline model for sepsis. This is due to the disruptive effect the parvovirus has on gut epithelial cells, promoting bacterial translocation from the intestinal lumen to the bloodstream, leading to bacteremia and SIRS (Petini et al. 2020; Gülersoy et al. 2023). Sepsis is considered as one of the complications of FPV infection, which leads to increased mortality. Therefore, the present study evaluated cats with panleukopenia infection and SIRS incidence was calculated in these patients, allowing for identification of potential risk and prognostic factors for SIRS.

Firstly, our main objective was to determine whether SIRS criteria is adequate for SIRS diagnosis in cats with panleukopenia virus infection. Moreover, we aimed to investigate potential risk factors for SIRS in cats, as well as prognostic factors, in order to aid clinicians in early SIRS detection, adapt treatment approaches and identify prognostic factors that would help develop realistic views on patient prognosis.

3.2. Materials and Methods

3.2.1. Inclusion Criteria

The animals enrolled in this study included all feline patients admitted to the Isolation and Biologic Containment Unit of the HEV-FMV-ULisboa with confirmed Panleukopenia Virus Infection, from October of 2015 to December of 2022. All animals had a complete blood count and basic biochemistry results, as well as registers of individual clinical exams upon admission and throughout their hospitalization, therapeutic approach and known outcome (death or discharge). The parvovirus infection diagnosis was determined by a positive result to either a PCR antigen detection test (from a fecal and/or plasma sample) or immunochromatography from a fecal sample. Animals with reports of recent vaccinations which could interfere with parvovirus infection diagnosis were excluded from the study.

All animals that did not fulfil the inclusion criteria or had non-infectious concomitant diseases were excluded from the study. For risk factor analysis purposes, cats that had

concomitant infectious diseases (Retrovirus, Feline Upper Respiratory Tract Disease and Gastrointestinal parasites) were included.

3.2.2. Data Recovery

As this study is of retrospective nature, all data concerning the patients was collected from the hospital's software, Guruvet® and Pet Universal©, as well as from the archives of the Isolation Unit. All clinical files were individually analyzed and the patients were submitted to both 1991 and 2001 SIRS criteria.

Basic patient information was collected, such as sex, age, weight, breed, reproductive status, vaccination status and environment (indoor/outdoor, cohabitation with other animals). The clinical parameters from the patients' physical exams that were included in the study were the animal's mental status, respiratory rate, heart rate, rectal temperature, mucous membrane color, CRT, pain or discomfort upon abdominal palpation, pulse, dehydration levels, alterations to palpable lymph nodes and presence of altered neurologic clinical signs. All of these parameters were evaluated upon admission as well as during hospitalization and their evolution was registered. Presence of diarrhea (bloody or not), vomiting, anorexia and hyporexia, concentrated urine emissions and coagulation dysfunctions were also registered and included for analysis. The analytical parameters that were retrieved from CBC included leucocyte, lymphocyte, neutrophil, monocyte, erythrocyte, hematocrit and platelet counts. The parameters retrieved from biochemistry included albumin, creatinine, urea, total protein, ALT and glycemia.

3.2.3. SIRS Classification

To determine whether systemic inflammatory response syndrome was occurring, each physical examination was evaluated and the respiratory rate, heart rate, rectal temperature, leucocyte count, CRT and mucosal membrane color were classified as being altered or not, according to the SIRS parameter references. As stipulated by the 1991 ACCP/SCCM Consensus Conference Committee, only cats with 3 or more altered parameters at a given moment were considered as fulfilling the SIRS 1991 criteria in the present study. In regards to the SIRS 2001 criteria, as proposed by Alves et al. (2020) based on the 2001 SCCM/ESCIM/ACCP/ATS/SIS International Sepsis Definitions Conference, these same parameters are also evaluated, yet only if the patient manifested an altered CRT and/or mucosal membrane color.

In order to increase the sensitivity of these criteria in this study, only cats that presented with two altered physical examinations during a period of 24 hours were classified as having SIRS. Furthermore, all cats were subjected to both 1991 SIRS criteria and 2001 SIRS criteria, to determine which of these is more indicative of a worser outcome. In 2001, the SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference proposed a list of

clinical signs that might occur during a systemic inflammatory response to infection that could be added to the 1991 SIRS criteria in order to increase specificity. In 2020, Alves et al. proposed the 2001 SIRS criteria based on this consensus, in which the SIRS criteria would only be applied if the patient had altered CRT or mucosal membrane color. The 2001 SIRS criteria were adapted and evaluated in this study.

3.2.4. Classification of Other Variables

3.2.4.1. Classification of Feline Upper Respiratory Tract Disease

The presence of Feline Upper Respiratory Tract Disease (URTD) was determined by a positive PCR result to any of the infectious agents or by a clinical score developed by Maximino (2021), adapted from Binns et al. (1999), based on the patients' clinical signs.

3.2.4.2. Age Classification

The "Age" variable was divided into three categories: "≤ 5 months", "5 months – 1 year" and "≥ 1 year). Those classified as "≤ 5 months" include all cats with 5 months or less; "5 months – 1 year" include those with more than 5 months and less than a year of age; "≥ 1 year" include patients with 1 year of age or more. This division was chosen due to the age at which cats usually have a serum decline in maternal antibodies and develop their own immune system with inoculation of vaccines, which is around 5 months of age (Sykes and Parrish 2023).

3.2.4.3. Vaccination Status Classification

The "Vaccination Status" variable was divided into "Vaccinated", "Incomplete" and "Not vaccinated". "Vaccinated" cats were patients with a correct primary vaccination, completed no earlier than 16 weeks of age, with annual reinforcements and up to date. "Incomplete" cats include those with an incomplete primary vaccination, a complete primary vaccination having terminated earlier than 16 weeks of age or cats that did not have annual reinforcements and/or were not up to date. "Not vaccinated" patients include those with no vaccine inoculation registration whatsoever.

3.2.4.4. Disseminated Intravascular Coagulation Classification

Each patient's clinical file was analyzed to determine whether they might have developed DIC during their hospitalization. Due to its difficult diagnosis, it was not possible to determine a positive diagnosis in any of the patients; therefore, these were classified as "Suspect" of DIC if they presented certain clinical signs indicative of a coagulopathy, such as presence of ecchymoses, petechiae, visible bruising or active hemorrhages as well as an analytical thrombocytopenia. Patients with none of the above were classified as "No".

3.2.4.5. Multicat House Classification

The patients' clinical history was analyzed and each patient was classified as living in a multicat house or not. "Multicat house" was considered when there were two or more cats living in the same space.

3.2.5. Statistical Analysis

The collected data was recorded on Microsoft® Office Excel 2023 Version 16.71. Subsequently, the data was then analyzed in R© Version 4.2.2 GUI 1.79, where the specific statistical tests were performed.

3.2.5.1. SIRS Criteria

The patients included in the study were determined as SIRS 1991 positive, SIRS 2001 positive or negative to either SIRS criteria. Following their classification, and in order to establish whether a correlation between each classification and the outcome exists, the results were subjected to Fisher's exact test, considering a 95% confidence interval. The mortality rate for cats with FP was also calculated.

3.2.5.2. Risk Factors for SIRS

In this section of the study, the totality of the patients (70) were considered and submitted to statistical analysis. Twenty-five of the 70 met the SIRS 2001 criteria and were, therefore, considered positive for SIRS. In order to determine potential risk factors for SIRS in cats infected with parvovirus, contingency tables were created associating the different variables with the presence or absence of SIRS and Chi-squared test or Fisher's exact test were performed to assess whether there was a correlation between them. Fisher's exact test results were chosen when the conditions for the Chi-squared test were not met. An 80% confidence interval was considered in order to explore associations between individual variables and SIRS diagnosis. The 80% confidence interval (CI) was chosen to identify potential associations that would not be considered in a 95% CI, and this allowed for a broader threshold to further include potential variables in the multivariate analysis.

All results that showed a *p*-value <0.2 were selected and further subjected to multivariate logistic regression analysis to identify the independent risk factors for SIRS in cats with FP. During the multivariate analysis, the focus shifted towards building a more selective and comprehensive logistic regression model that accounted for the association between variables while adjusting for potential confounders. Therefore, in this instance, a 95% CI was chosen.

3.2.5.3. Prognostic Factors for Cats with SIRS

To assess the predictive significance of certain clinical signs and hematological values in cats for mortality or survival to discharge, cats with SIRS were selected, creating a new study sample of 25 patients. From this new sample, a correlation was tested between clinical

signs or blood test results and the outcome, using the Fisher's exact test, considering a 95% confidence interval. A multivariate analysis of these factors was not possible due to the reduced sample size.

3.3. Results

3.3.1. SIRS

A cohort of 70 panleukopenia-positive cats admitted to the Isolation and Biologic Containment Unit of the HEV-FMV-UL was evaluated. Fisher's exact test was applied to 1991 and 2001 SIRS criteria to establish whether there is a correlation with the outcome (Table 2 and Table 3). Both the SIRS 1991 and 2001 criteria demonstrated *p* values inferior to 0.05 in association with the outcome and were considered statistically significant.

As, according to Table 3, the SIRS 2001 criteria have a smaller *p*-value than the SIRS 1991 criteria and were suggested as a way to increase specificity, these were chosen for the remainder of the study as our "true SIRS" and used in all statistical analyses from here on.

Table 2 – Fisher's Exact Test Results: association SIRS 1991 and 2001 with outcome (Death or Survival to discharge)

	Death	Survive to Discharge	*p*-value
SIRS 1991			0.0001675
Yes	16	13	
No	5	36	
SIRS 2001			0.00008578
Yes	15	10	
No	6	39	

Legend: SIRS – Systemic Inflammatory Response Syndrome

Table 3 - Fisher's exact test for correlation of both SIRS criteria and the outcome (Death or Survival to discharge).

	p-value	Odds Ratio	95 % CI
SIRS 1991	0.00017	8.54	2.40-36.14
SIRS 2001	0.00008	9.35	2.64-37.87

Legend: CI - Confidence interval; SIRS – Systemic Inflammatory Response Syndrome

3.3.2. Risk Factors for SIRS in Cats with Panleukopenia

3.3.2.1. Sample Characterization

For this section of the study, a total sample of 70 cats which were hospitalized in the Isolation Unit of the HEV-FMV-ULisboa with a positive diagnosis for FP were included.

Of the 70 cats that were included, the sex distribution was as follows: 18 (25.7%) were neutered females, 16 (22.9%) were intact females, 17 (24.3%) were neutered males and 19 (27.1%) were intact males. Concerning the breed, 63 (90.0%) did not have a specified breed, 2 were Maine Coon, 2 were Persian, 1 was Savannah, 1 was Scottish Fold, and 1 was Sphynx. In regards to age, 22 (31.4%) were aged 5 months or less, 25 (35.7%) were over 5 months and under 1-year-old and 23 (32.8%) were 1 year old or over. As for vaccination status, most cats, 44 (62.8%) were not vaccinated, with only 4 (5.7%) being correctly vaccinated and 22 (31.4%) with an incomplete vaccination record. As cats with concomitant infectious diseases were not excluded from the study sample, these were also analyzed but are a minority (N=8), where 2 (2.8%) had Feline Upper Airway Disease, 4 (5.7%) had retrovirus infection (3 FIV and 1 FeLV) and 2 (2.8%) had gastrointestinal parasites. The remaining 62 (88.6%) had no concomitant disease. Most results can be consulted in Tables 4 and 5. Environment factors were also analyzed, such as access to exterior and cohabitation with other cats. Twenty-four cats out of 70 lived in a multicat house whereas only 28.5% had access to the exterior.

Upon admission, 36 cats (51.4%) were hypoalbuminemic and 2 (2.86%) had increased albumin values. As for glycemia, 30 (42.9%) were hyperglycemic, 11 (15.7%) were hypoglycemic, and the remaining 29 (41.4%) had normal serum glucose values. Seventeen (24.3%) cats had increased ALT activities upon admission and only 1 (1.43%) had increased creatinine values. The measurement of serum total protein was only available for 60 cats. Among them, 22 (36.7%) cats exhibited hypoproteinemia and 5 (8.3%) displayed increased values. On the other hand, urea measurement was only performed on 65 cats, where 7 (10.8%) had increased urea values. As for hemogram values, 47 (67.1%) cats had leukopenia on admission and in only 4 of these, this was due exclusively to lymphopenia, demonstrating normal neutrophil counts. Seven of the leukopenic cats had normal lymphocyte counts (with decreased neutrophil counts) and the remaining 36 showed both decreased values in lymphocyte and neutrophil counts. Fifty-four (77.1%) cats had thrombocytopenia upon admission, whereas 16 (22.9%) displayed normal platelet counts.

Table 4 – Sample characterization of cats subjected to risk factor for SIRS analysis

Gender	N (%)	Age	N (%)	Vaccination status	N (%)	Concomitant diseases	N (%)
Male	38 (54.3)	< 5 months	22 (31.4)	Vaccinated	4 (5.7)	URTD	2 (2.8)
Female	32 (45.7)	5 months – 1 year	25 (35.7)	Incomplete	22 (31.4)	Retrovirus	4 (5.7)
		> 1 year	23 (32.8)	Not vaccinated	44 (62.8)	GI parasites	2 (2.8)
						None	62 (88.6)

Legend: URTD - Upper Respiratory Tract Disease; FIV - Feline Immunodeficiency Virus; FeLV - Feline Leukemia Virus; GI = gastrointestinal; N – number of patients; SIRS – Systemic Inflammatory Response Syndrome

As for treatment options (Table 5), 25 (35.7%) cats had placement of a nasoesophageal feeding tube and only three (4.3%) did treatment with interferon. In regards to blood product transfusions, 20 (28.6%) had transfusions, where 18 (25.7%) had fresh frozen plasma (FFP) transfusions, 1 (1.43%) had erythrocyte concentrate (EC) transfusion and 1 (1.43%) had both FFP and EC transfusions.

Table 5 – Sample characterization: treatments performed on cats with FP diagnosis

Feeding Tube	N (%)	Blood product transfusion	N (%)	Type of Transfusion	N (%)	Interferon	N (%)
Yes	25 (35.7)	Yes	20 (28.6)	FFP	18 (25.7)	Yes	3 (4.3)
No	45 (64.3)	No	50 (71.4)	EC	1 (1.43)	No	67 (95.7)
				Both	1 (1.43)		
				None	50 (71.4)		

Legend: % - percent; EC - Erythrocyte Concentrate; FFP - Fresh Frozen Plasma; FP – Feline Panleukopenia; N – number of patients

Regarding the outcome, 21 (30.0%) cats died whilst 49 (70.0%) survived to discharge, with a mortality rate of 30% in cats with FPV infection. Of the 21 cats that died, 16 (76.2%) had fulfilled the SIRS criteria 1991 and 15 (71.4%) cats fulfilled the SIRS criteria 2001. However, 10 cats fulfilled the SIRS criteria 2001 and survived.

By subjecting all patients to both SIRS 1991 and 2001 criteria, it was concluded that 29 (41.4%) of all patients fell under the original SIRS 1991 criteria whereas only 25 (35.7%) fulfilled the SIRS 2001 criteria, revealing a SIRS rate of 35 to 41% in cats with viral

panleukopenia. Therefore, only 4 of the cats that were classified as fulfilling the SIRS 1991 criteria did not have an abnormal CRT or mucosa color.

3.3.2.2. Risk Factors for SIRS

3.3.2.2.1. Univariate Analysis

A univariate analysis was performed on all patients included in the study, in order to assess a possible association between potential risk factors and the development of SIRS. The Chi-squared test and Fisher's exact test were used to compare the categorical variables and an 80% confidence interval was considered. As shown in Table 6, the association between breed, gender, reproductive status, age, access to exterior, multicat house environment, presence of infectious concomitant diseases, fresh frozen plasma transfusion, treatment with interferon, application of feeding tube, increased creatinine, increased ALT, leukopenia, neutropenia, erythrocyte count and thrombocytopenia with SIRS, were not considered statistically significant, all demonstrating *p*-values above 0.2. On the other hand, vaccination status, weight, the occurrence of blood products transfusions, hypoalbuminemia, hypoproteinemia, hypoglycemia, lymphopenia and hematocrit measure demonstrated statistical significance when associated with the occurrence of SIRS, with *p*-values < 0.2.

Table 6 – Chi-squared test and Fisher's exact test results: association between potential risk factors and the development of Systemic Inflammatory Response Syndrome

Variables	SIRS (%)	No SIRS (%)	*p*-value
Breed			0.534
Maine Coon	0 (0)	2 (4.44)	
Persian	0 (0)	2 (4.44)	
Savannah	0 (0)	1 (2.22)	
Scottish Fold	0 (0)	1 (2.22)	
Sphynx	1 (4)	0 (0)	
Undefined	24 (96)	39 (86.67)	
Gender			0.224
Male	16 (64)	22 (48.9)	
Female	9 (36)	23 (51.1)	
Neutered			0.803
No	13 (52)	22 (48.9)	
Yes	12 (48)	23 (51.1)	
Age			0.219
< 5 months	11 (44)	11 (24.4)	
5 months – 1 year	8 (32)	17 (37.8)	
> 1 year	6 (24)	17 (37.8)	

Variables	SIRS (%)	No SIRS (%)	*p*-value
Weight			**< 0.01**
< 2 Kg	11 (44)	7 (15.6)	
2-5 Kg	14 (56)	32 (71.1)	
> 5 Kg	0	6 (13.3)	
Access to exterior			0.211
No	14 (56)	32 (71.1)	
Yes	8 (32)	12 (26.7)	
Unknown	3 (12)	1 (2.2)	
Multicat house			0.409
Yes	18 (72)	28 (62.2)	
No	7 (28)	17 (37.8)	
Vaccination Status			0.160
Vaccinated	0 (0)	4 (8.89)	
Not vaccinated	19 (76)	25 (55.56)	
Incomplete	6 (24)	16 (35.55)	
Infectious concomitant diseases			1
Yes	3 (12)	5 (11.1)	
No	22 (88)	40 (88.9)	
Infectious concomitant diseases			0.222
URTD	0 (0)	2 (4.4)	
Retroviruses	1 (4)	3 (6.7)	
Gastrointestinal parasites	2 (8)	0 (0)	
None	22 (88)	40 (88.9)	
Blood Products Transfusion			0.115
Yes	10 (40)	10 (22.2)	
No	15 (60)	35 (77.8)	
Frozen Fresh Plasma Transfusion			0.214
Yes	9 (36)	10 (22.2)	
No	16 (64)	35 (77.8)	
Feeding Tube			0.281
Yes	11 (44)	14 (31.1)	
No	14 (56)	31 (68.9)	
Interferon			0.289
Yes	2 (8)	1 (2.2)	
No	23 (92)	44 (97.8)	

Variables	SIRS (%)	No SIRS (%)	*p*-value
Hypoalbuminemia upon admission			**<0.05**
Yes	17 (68)	19 (42.2)	
No	8 (32)	26 (57.8)	
Hypoproteinemia upon admission			**<0.05**
Yes	11 (57.9)	11 (26.8)	
No	8 (42.1)	30 (73.2)	
Hypoglycemia upon admission			**<0.0001**
Yes	10 (40)	1 (2.2)	
No	15 (60)	44 (97.8)	
Hyperglycemia upon admission			0.388
Yes	9 (36)	21 (46.7)	
No	16 (64)	24 (53.3)	
Increased creatinine upon admission			1
Yes	0 (0)	1 (22.2)	
No	25 (100)	44 (97.8)	
Increased ALT upon admission			0.533
Yes	5 (20)	12 (26.7)	
No	20 (80)	33 (73.3)	
Leucopenia upon admission			0.240
Yes	19 (76)	28 (62.2)	
No	6 (24)	17 (37.8)	
Neutropenia upon admission			0.315
Yes	18 (72)	27 (60)	
No	7 (28)	18 (40)	
Lymphopenia upon admission			**<0.1**
Yes	19 (76)	25 (55.6)	
No	6 (24)	20 (44.4)	
Thrombocytopenia upon admission			0.671
Yes	20 (80)	34 (75.6)	
No	5 (20)	11 (24.4)	

Variables	SIRS (%)	No SIRS (%)	*p*-value
Hematocrit measure upon admission			0.123
Increased	1 (4)	9 (20.0)	
Decreased	5 (20)	5 (11.1)	
Normal	19 (76)	31 (68.9)	
Erythrocyte measure upon admission			1
Increased	0 (0)	0 (0)	
Decreased	2 (8)	3 (6.8)	
Normal	23 (92)	41 (93.2)	

Legend: % - percent; URTD – Upper Respiratory Tract Disease; Kg – kilogram; SIRS – systemic inflammatory response syndrome; ALT – alanine transaminase

3.3.2.2.2. Multivariate Analysis

Following the univariate analysis, multivariate logistic regression analysis was performed to identify the independent risk factors for SIRS. The multivariate analysis results are shown in Table 7. The variables associated with SIRS diagnosis in the univariate analysis were introduced in the multivariate model (stepwise selection). Treatment of the patients with blood product transfusions were found as a non-independent risk factor, with a *p*-value > 0.05. Due to the confounding nature of Serum Total Protein values and Serum Albumin values, Total Protein results were excluded from the model. However, the presence of hypoproteinemia remains to be a statistically significant risk factor for SIRS in this study. As shown in Table 7, hypoalbuminemia upon admission (*p*=0.027, OR=3.22; CI, 1.19-9.84), hypoglycemia upon admission (*p*=0.0023, OR=12.14; CI, 3.14-103.58) and lymphopenia upon admission (p=0.0476, OR=2.88; CI, 1.06-8.91) were identified as independent risk factors for SIRS in cats with panleukopenia virus infection. Weight was not considered an independent risk factor for SIRS, with *p*-values > 0.05.

Table 7 - Multivariate logistic regression analysis results of risk factors for SIRS.

Factors	Estimate	Std Error	Z value	*p*-value	OR	95 % CI
Weight						
< 2 Kg	-1.18	0.73	-1.61	0.10697	0.31	0.07-1.28
> 5 Kg	17.11	1544.03	0.01	0.99116	2704.08×10^{4}	2.53×10^{-56}-NA
Hypoalbuminemia upon admission	1.17	0.53	2.21	0.02706	3.22	1.19-9.84
Hypoglycemia upon admission	2.50	0.82	3.05	0.00228	12.14	3.14-103.58
Lymphopenia upon admission	1.06	0.53	1.98	0.04757	2.88	1.06-8.91

OR - Odds ratio; CI - Confidence interval; Kg - Kilogram; SIRS - Systemic Inflammatory Response Syndrome; Std - standard

3.3.3. SIRS Characterization and Prognostic Factors

3.3.3.1. Sample Characterization

For this section of the study, only the SIRS positive cats (25) which were hospitalized in the Isolation Unit of the HEV-FMV-ULisboa were analyzed.

Of these 25 cats, 15 (60%) died, whilst 10 (40%) survived to discharge. Concerning sex, a majority of 16 (64%) were male and 9 (36%) were female and, of the ones with a negative outcome, 9 were male and 6 were female. None of the cats which were considered as SIRS positive were correctly vaccinated, with 19 (76%) not having had a single vaccine and 6 (24%) being incorrectly vaccinated. The median age of the cats classified as SIRS was of 6 months (0,5 years) and mean of 1.2767±1.8189 although age did not follow a normal distribution, according to Shapiro-Wilk test (Appendix 4) with 15 cats being younger than 6 months, accounting for 60% (Appendix 3 and 4). Following our classification, 11 (44%) were aged under 5 months, 8 (32%) were over 5 months old and under 1 year, and 6 (24%) were 1 year old or above. Only 3 cats had concomitant infectious diseases; 1 with feline immunodeficiency virus (FIV) and 2 with gastrointestinal parasites.

Upon admission, 19 (76%) cats had leukopenia, whereas only 3 (12%) had leukocytosis. Some of the patients displayed such an accentuated low leukocyte count that the differential leukocyte count was not possible. As for neutrophil counts, 18 (72%) cats had neutropenia and 3 had neutrophilia, whereas 19 (76%) had lymphopenia, 5 (20%) had lymphocytes within normal range and only 1 (4%) presented with lymphocytosis. Regarding eosinophils, basophils and monocytes, all cats had normal counts except for one which

displayed monocytosis. Considering the hemogram, most (92%) had normal erythrocyte count, and only 2 (8%) demonstrated erythropenia. As for the hematocrit, 19 (76%) cats had a hematocrit within the normal range, 5 (20%) had a low hematocrit and only 1 (4%) had an increased value. Regarding platelets, 20 (80%) cats showed thrombocytopenia and 5 (20%) showed normal platelet count. Five cats had non-regenerative anemia upon admission, and only one normalized its values during hospitalization, whereas the remaining 4 worsened. However, during hospitalization, an additional 6 cats developed anemia.

Analyzing the biochemical results, 17 out of 25 cats showed hypoalbuminemia upon admission, and one showed increased values of serum albumin. Of the 19 cats which had available total protein values, 11 (57.9%) were hypoproteinic upon admission. Five (20%) cats showed an increase in ALT activities upon admission and as for glycemia, 9 (36%) displayed hyperglycemia upon admission, 10 (40%) displayed hypoglycemia and the 6 (24%) remaining were normoglycemic. Regarding creatinine and urea, all cats displayed diminished values or within normal range, except for two cats with increased urea values. Lactate values were only measured in 4 cats and were increased in all.

Among the 25 cats included in this section of the study, a range of clinical signs were observed. The most common clinical signs reported by the owners were anorexia or hyporexia (100%), vomiting (64%), diarrhea (60%), and depression (60%). Clinical signs at admission and throughout hospitalization were observed by the staff and treatments were analyzed. All of these results can be consulted in Table 8. As for physical exam findings at admission, 15 (60%) cats were dehydrated, 8 (32%) had altered mucous membrane color (pale or icteric), 1 had altered CRT, 2 had altered pulse, 3 had presence of lymphadenomegaly of the palpable lymph nodes, 10 (40%) were hyperthermic, 5 (20%) were hypothermic and 9 (36%) showed discomfort or pain at abdominal palpation. Mental status at admission was assessed, where 10 (40%) cats were alert, and 15 (60%) had an abnormal mental status. All cats that were discharged became alert during hospitalization, whilst most cats that died maintained an abnormal mentation, except for one who was alert. During hospitalization, 12 cats recovered their appetite and 19 ceased vomiting. Fourteen cats maintained or developed dehydration during hospitalization, and of these only 3 were survivors. Of the non-survivors, all 15 had altered mucous membrane color during hospitalization and maintained diarrhea. As for the survivors, only two recovered from the diarrhea during hospitalization, whereas 8 did not. Sixteen of all cats had visible blood in their feces, with similar values among survivors and non-survivors. Five cats maintained discomfort or pain at abdominal palpation and 5 developed oral ulcers. Only 5 cats showed neurological clinical signs during hospitalization, and 10 developed concentrated urine.

In regards to treatment, most cats underwent the same protocol treatment approach. All 25 cats (100%) received intravenous Ringer's Lactate and were treated with amoxicillin and

clavulanic acid. Twenty cats (80%) were also given metronidazole and only 14 (56%) were treated with marbofloxacin. As for fluid supplementation, 17 (68%) were supplemented with glucose (G30 at 2.5%) and 15 (60%) were supplemented with potassium chloride (KCl) at maintenance values.

Table 8 – Fisher's exact test results: association between clinical signs, hematological results and treatment and outcome

Variables	Death	Discharge	*p*-value	OR	CI
Anorexia or hyporexia at admission			1	0	0-Inf
Yes	15	10			
No	0	0			
Anorexia or hyporexia during hospitalization			0.0009826	29.26	2.59-1680.99
Yes	12	1			
No	3	9			
Abnormal mental status at admission			1	1	0.14-6.69
Yes	9	6			
No	6	4			
Abnormal mental status during hospitalization			0.000003365	Inf	9.75-Inf
Yes	14	0			
No	1	10			
Dehydration upon admission			1	1	0.14-6.69
Yes	9	6			
No	6	4			
Dehydration during hospitalization			0.04862	5.89	0.84-55.20
Yes	11	3			
No	4	7			
Altered mucous membrane color upon admission			0.6668	0.56	0.07-4.20
Yes	4	4			
No	11	6			
Altered mucous membrane color during hospitalization			0.4	Inf	0.04-Inf
Yes	15	9			
No	0	1			
Altered CRT upon admission			0.4	0	0-26.00
Yes	0	1			
No	15	9			
Altered CRT during hospitalization			0.229	3.33	0.44-42.66
Yes	7	2			
No	8	8			

Variables	Death	Discharge	*p*-value	OR	CI
Altered pulse at admission			0.15	0	0-3.44
Yes	0	2			
No	15	8			
Altered pulse during hospitalization			0.6146	3.13	0.25-178.57
Yes	4	1			
No	11	9			
Diarrhea upon admission			1	1	0.14-6.69
Yes	9	6			
No	6	4			
Diarrhea during hospitalization			0.15	Inf	0.29-Inf
Yes	15	8			
No	0	2			
Diarrhea evolution during hospitalization			0.15	Inf	0.29-Inf
Improved	0	2			
Maintained or worse	15	8			
Blood in stool			0.6913	0.65	0.08-4.56
Yes	9	7			
No	6	3			
Vomiting upon admission			1	1.32	0.18-9.29
Yes	10	6			
No	5	4			
Vomiting during hospitalization			1	1.43	0.16-19.58
Yes	4	2			
No	11	8			
Lymphadenomegaly upon admission			0.5435	0.30	0.0045-6.64
Yes	1	2			
No	14	8			
Neurological signs			1	1	0.07-10.96
Yes	3	2			
No	12	8			
Oral ulcers			0.3577	0.37	0.25-4.10
Yes	2	3			
No	13	7			
Concentrated urine emission			0.6785	1.95	0.29-16.67
Yes	7	3			
No	8	7			
Fever upon admission			1	1	0.15-7.09
Yes	6	4			
No	9	6			
Hypothermia upon admission			0.6146	3.13	0.25-178.57
Yes	4	1			
No	11	9			

Variables	Death	Discharge	*p*-value	OR	CI
Discomfort or pain at abdominal palpation upon admission			1	1.32	0.18-9.29
Yes	5	4			
No	10	6			
Discomfort or pain at abdominal palpation during hospitalization			1	1	0.07-10.96
Yes	3	2			
No	12	8			
DIC			0.5	Inf	0.12-Inf
Suspect	2	0			
No	13	10			
Amoxicilin with clavulanic acid treatment			0.25	Inf	0.28-Inf
Yes	15	10			
No	0	0			
Marbofloxacin treatment			0.6968	0.68	0.10-4.44
Yes	9	5			
No	6	5			
Metronidazole treatment			1	1	0.09-14.53
Yes	12	8			
No	3	2			
Supplementation with glucose			0.6668	0.56	0.07-4.20
Yes	11	6			
No	4	4			
Supplementation with KCL			1	1	0.15-7.09
Yes	9	6			
No	6	4			
Crystalloid bolus			0.6146	0.30	0.02-2.30
Yes	4	1			
No	11	9			
Glucose bolus			0.6592	0.51	0.04-4.28
Yes	5	2			
No	10	8			
Leukopenia upon admission			0.1753	4.06	0.44-56.96
Yes	13	6			
No	2	4			
Neutropenia upon admission			0.07523	5.96	0.70-82.40
Yes	13	5			
No	2	5			
Lymphopenia upon admission			0.02253	12.37	1.04-704.29
Yes	14	5			
No	1	5			
Hypoalbuminemia upon admission			0.6668	1.79	0.24-13.85
Yes	11	6			
No	4	4			

Variables	Death	Discharge	*p*-value	OR	CI
Hypoproteinemia upon admission			1	0.60	0.04-6.14
Yes	7	4			
No	6	2			
Hypoglycemia upon admission			0.6785	1.98	0.29-16.67
Yes	7	3			
No	8	7			
Hyperglycemia upon admission			0.3973	0.38	0.05-2.64
Yes	4	5			
No	11	5			
Increased creatinine values upon admission			1	0	0-Inf
Yes	0	0			
No	15	10			
Increased ALT values upon admission			0.3577	0.38	0.02-4.10
Yes	2	3			
No	13	7			

Legend: ALT – alanine transaminase; CI – confidence interval; CRT – capillary refill time; DIC – Disseminated Intravascular Coagulation; KCL – potassium chloride; OR – odds ratio

The results for the Fisher's exact test associating the different variables with the outcome are displayed on Table 9. According to the results, the association of the following variables with mortality are all statistically significant with *p*-values inferior to 0.05: development or maintenance of dehydration during hospitalization (*p*=0.0486, OR=5.89; CI, 0.84-55.20), anorexia or hyporexia during hospitalization (*p*=0.0009, OR=29.26; CI, 2.59-1680.90), abnormal mental status during hospitalization (*p*=0.000003, OR=Inf; CI, 9.7536-Inf) and lymphopenia upon admission (*p*=0.02253, OR=12.37; CI, 1.04-704.29).

Table 9 – Fisher's exact test results of prognostic factors for cats with Systemic Inflammatory Response Syndrome (SIRS)

Variables	Death	Discharge	*P*-value	OR	CI
Anorexia or hyporexia during hospitalization			< 0.001	29.26	2.59-1680.99
Yes	12	1			
No	3	9			
Abnormal mental status during hospitalization			< 0.00001	Inf	9.75-Inf
Yes	14	0			
No	1	10			
Dehydration during hospitalization			< 0.05	5.89	0.84-55.20
Yes	11	3			
No	4	7			
Lymphopenia upon admission			< 0.05	12.37	1.04-704.29
Yes	14	5			
No	1	5			

Legend: CI – confidence interval; OR – odds ratio; SIRS – systemic inflammatory response syndrome

3.4. Discussion

Sepsis and non-infectious SIRS continue to be major causes of death in ICUs and are important causes of mortality in humans, dogs and cats (Babyak and Sharp 2016). In recent years, there has been a notable surge in research focused on sepsis and SIRS leading to the rapid emergence of new evidence and a greater understanding of sepsis pathophysiology. The development of diagnostic and treatment guidelines by the SCC, for example, has significantly aided in the ongoing battle that is sepsis management and has decreased morbidity and mortality rates (Babyak and Sharp 2016; Evans et al. 2021). However, it should be noted that a substantial portion of what is known about sepsis and SIRS remains inadequately supported by empirical evidence, leaving many aspects yet to be investigated and explored, especially in veterinary medicine. Studies concerning sepsis and SIRS in feline patients, specifically, are notably limited and this emphasizes the need for more research. Few studies have evaluated risk factors and prognostic factors in cats with SIRS and sepsis and, as such, this retrospective study was conducted in order to shed some light on the matter.

As explained previously, the definition of sepsis determined by the SOFA score has not yet been adapted to veterinary medicine. Alves et al. (2020) proposed the 2001 SIRS criteria, based on the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference, where the 1991 SIRS criteria would only be applied if the CRT or mucous membrane color were abnormal. This is thought to have increased specificity slightly but given that SIRS can be triggered by infectious and non-infectious conditions, such as burns and pancreatitis (Levy et al. 2003), it remains as having low sensitivity and specificity.

3.4.1. SIRS criteria

In this study, seventy cats with confirmed parvovirus infection were included and assessed using both 1991 and 2001 SIRS criteria. As no blood cultures were performed, it was not possible to affirm that these cats had developed sepsis and hence they are only regarded as having developed or not SIRS, according to each criteria (1991 and 2001). Statistically significant associations of both 1991 and 2001 SIRS criteria with mortality were observed, yielding *p*-values of 0.00017 and 0.00008, respectively. Some studies have evaluated the SIRS criteria prediction of mortality in cats and dogs and show conflicting results. Declue et al. (2011) reported no association between the 1991 SIRS criteria and the negative outcome in cats, which contradicts the results in our study. This discrepancy between results may be due to the fact that in our study, only cats with 2 or more altered physical exams within a 24-hour period, with 3 or more SIRS criteria in each physical exam, were considered as SIRS positive. This assumption was made in an attempt to increase specificity of the SIRS criteria. However, this difference in results also highlights the urgent need of a consensus defining SIRS and sepsis diagnosis in cats.

According to the results presented in this study (Tables 2 and 3), cats that fulfilled the 2001 SIRS criteria were 9 times more likely to die than cats that did not, and 8 times more likely to die when subjected to the 1991 criteria. These results show that both criteria may be used in a clinical scenario. Six patients with parvovirus infection died and were not considered SIRS positive; however, the cause of death was not determined. Two of the cats that died without fulfilling SIRS criteria had concomitant infectious diseases other than panleukopenia virus, namely calicivirus infection and FeLV. However, the calicivirus infected cat did have a single altered physical examination, which imposes a dilemma, as the strategy of only considering two altered physical exams may overlook possible SIRS cases. Moreover, the other patient did not have evidence of any abnormal physical examination determined by the SIRS criteria. This may indicate either other infectious complications inherent to those diseases or simply that physical examination timings were inopportune and failed to detect SIRS with its criteria.

3.4.2. Risk Factors for SIRS in cats

The identification and understanding of risk factors for SIRS and sepsis is crucial in veterinary medicine, in order to enhance early detection and begin a timely aggressive therapeutic approach, which has been shown to decrease mortality (Levy et al. 2018). This may, in turn, allow for a more realistic prognosis. In this study, potential risk factors for SIRS development in cats were explored.

Many potential risk factors were analyzed, and hypoglycemia, hypoalbuminemia, hypoproteinemia and lymphopenia upon admission showed a significant statistical association with SIRS and were, therefore, considered as independent risk factors for SIRS ($p<0.05$).

In regards to age, it has been shown in humans that an increased age is considered to be a risk factor for SIRS and sepsis development (Iskander et al. 2013; Mayr et al. 2014). Reports suggesting age-associated changes in the inflammatory response of dogs have also been made (Deitschel et al. 2010). In this study, however, age was not an independent risk factor for SIRS development ($p=0.2193$). The contradiction between these findings and the existing literature can be explained by the age structure of our study sample, where most patients were less than one year old, due to the underlying disease which mainly affects kittens at around 5 months of age. Therefore, the sample did not have a varied array of age groups and this may influence the final results.

Although vaccination status showed an association with SIRS in the initial univariate analysis ($p<0.2$), it was discarded in the choosing of the most adequate logistic regression model, due to the lack of statistical significance at the chosen significance level. It would be expected that cats with no vaccination would have more inadequate host responses and potentially be more susceptible to SIRS and sepsis development (Sykes and Parrish 2023). There are some possible explanations for the absence of association with SIRS diagnosis, although this has not been investigated previously in feline populations. Most of these cats came from other medical centers and, therefore, vaccination protocols differ considerably between individuals due to the opinion and personal criteria of each clinician, making it difficult to determine whether an adequate vaccination protocol was carried out. Nonetheless, maternally derived antibodies may also interfere with vaccination and these have been reported to persist until 20 weeks of age in some cases, so even in cases where booster vaccines are administrated at 16 weeks, MDAs may still interfere (Sykes and Parrish 2023). It is also important to note that, as it is retrospective in nature, this study has limitations associated with data collection, namely the veracity of owner-reported information, as well as the accuracy of the archival data. Nonetheless, the absence of association may in fact demonstrate the lack of influence that vaccinations may have in SIRS and sepsis onset, as the serologic and immunologic response to vaccines may only aid in a first instance of pathogen clearance and not influence the systemic inflammation which is characteristic of both in SIRS and sepsis.

Breed was also not associated with the outcome in the present study ($p=0.534$). Although breed influences have not been studied in cats, it has been shown that certain dog breeds susceptible to parvoviral enteritis demonstrate different inflammatory responses from other dogs, with an increased production of TNF-α (Nemzek et al. 2007). In fact, the decreased genetic diversity that is consequence of selective breeding may have an influence on the

immune response of each animal, and animals of certain breeds may be more susceptible to infection. The results concerning breed in this study may be explained by the small sample size, the decreased number of cats with specific breeds that were included and the small diversity of breeds. Thus, other studies with a larger sample size and bigger breed ratios should be performed in the future.

In this study, sex was also not considered an independent risk factor. Sex as a risk factor has been a matter of debate, with many differing opinions. Some human studies have established an increased prevalence of SIRS and sepsis in male patients, and other authors have discredited these findings (Combes et al. 2009; Iskander et al. 2013; Angele et al. 2014). In experimental sepsis in animals, however, females have been reported to have a lower tendency for SIRS and display an inherent survival advantage in polymicrobial sepsis (Drechsler et al. 2012; Iskander et al. 2013). Although no significant statistical association was made between gender and SIRS diagnosis (p=0.224), an increased prevalence was observed in male cats, with 16 males compared to 9 females. These findings corroborate most human and animal studies, although the underlying mechanisms behind this disparity between genders is unknown. Some proposed explanations include the role of sex hormones that may influence the risk of developing SIRS, as well as sex-related genetic polymorphisms (Angele et al. 2014).

Cats with concurrent infectious diseases were not excluded from this study, as viral infections, immune system defects and parasitism have been defined as risk factors for sepsis in kittens (Hoskins 1993). Additionally, certain comorbidities have been determined as relevant risk factors for sepsis onset in human patients, such as immunosuppressive diseases (HIV) or treatment with immunosuppressants (Iskander et al. 2013; Mayr et al. 2014; Cecconi et al. 2018). Some studies have shown that the onset of systemic inflammation in patients with HIV differs from a healthy person, as the effective immune response is affected, with dysfunction of many cells such as T-cells, neutrophils and macrophages. This abnormal immune response may increase patient susceptibility for infection and, additionally, exacerbate the already dysregulated inflammatory response characteristic of sepsis (Huson et al. 2015). In cats, both FIV and FeLV infections cause immunosuppression and innate and adaptive cell dysfunction, although differences in the inflammatory response are still not well understood. It is known, however, that there is a decrease in production of some proinflammatory cytokines and an increase in TNF-α (Beatty and Sykes 2023; Hartmann et al. 2023). Nevertheless, a similar response would be expected in cats with retrovirus infection, and presence of these infectious diseases could potentially be risk factors for SIRS and sepsis development. In this study, however, only 12 cats had concomitant infectious diseases, and none were determined as risk factors. These results can be explained by the small sample size that was analyzed and the even smaller number of cats with infectious diseases. As mentioned previously, parasitism has

also been identified as a risk factor (Hoskins 1993), so it would be expected for the patients with parasites in this study group to have developed SIRS. In fact, only two cats had evidence of gastrointestinal parasites, and both were diagnosed as SIRS positive. However, naturally an incidence of 2/70 cats is not a sufficient sample to conclude any findings.

Concerning weight as a potential risk factor, interesting findings were observed in this study. In the univariate analysis, an increase in weight demonstrated a statistically significant association with SIRS diagnosis, as indicated by a confidence interval of 80%, suggesting a potential relationship. However, in the multivariate analysis, weight did not retain its association with SIRS and thus was discredited as an independent risk factor. The multivariate analysis, conducted with a higher IC of 95%, revealed that the other variables in the logistic regression model were more strongly associated with SIRS diagnosis and confounded the relationship between weight and SIRS slightly. This discrepancy may, thus, be attributed to the presence of confounding factors which may have masked the real influence of weight on SIRS susceptibility. It is important to note that the results concerning weight in the multivariate analysis should be carefully interpreted, as the “NA” in the 95% CI signifies that the upper limit of the confidence interval could not be calculated, most probably due to the small number of cats in that particular category. Therefore, this imparts a considerable level of uncertainty regarding the association between weight and SIRS, and further investigations with a larger and more comprehensive dataset should be performed to obtain more reliable results. Although obesity and its effects on sepsis susceptibility are not well understood, there is evidence of obesity being correlated with increased susceptibility to infection and complications in humans (Mayr et al. 2014; Kalani et al. 2020). Further research is needed to explore the potential interaction between weight, obesity and SIRS in a feline population, accounting for possible confounders, to obtain a more comprehensive understanding of these complex connections.

As for other variables evaluated in this study, such as reproductive state, multicat house and access to exterior none correlated with SIRS, therefore do not qualify as risk factors. In a report on feline sepsis patients with pyothorax, the environment specificities were considered risk factors for SIRS development, where cats with access to the outdoors presented an increased risk of developing SIRS (Waddell et al. 2002). Additionally, multicat environments were also conducive for SIRS development. In fact, Hoskins et al. (1993) had also reported some environmental factors that may be influencing susceptibility, such as increased number of cats, humidity and temperature, sanitation and stress. In this study, we evaluated whether the patients lived in a multicat house and whether there was access to the exterior and it was determined that neither factor contributed to an increase in risk of SIRS. This is contrary to what was reported by Waddell et al. (2002), most probably because the causes of pyothorax in that study were due to bite wounds or cat fights that occur mainly in the street. Therefore,

the access to the exterior in that case was associated with the primary cause of pyothorax, which is one of the main causes of sepsis in cats (Costello 2015; Silverstein and Otto 2023). However, in FPV infections, the transmission occurs via direct contact with viral particles in feces and fomites, therefore infection is possible both outdoors and indoors, especially in overcrowded catteries where disinfection of the premises and vaccination is usually inadequate. This may explain the absence of association between the outdoors and SIRS development. On the other hand, it would be expected that stress would influence the individual's immune system and in a multicat environment, as reported, cats may be more susceptible to infection and SIRS development. These findings were not reported in this study, where there was no association with SIRS diagnosis.

Similar to vaccination status, hematocrit values were also initially considered as potential risk factors but were removed from the final regression model. This might be due to lack of statistical significance or to confounding factors that influenced the initial statistical association. Anemia is a common finding in cats with sepsis and, therefore, may be indicative of onset of SIRS and could show statistical significance when associated with SIRS diagnosis (Hoskins 1993; Brady et al. 2000; Costello et al. 2004; Klainbart et al. 2017). In the present study, however, most cats presented normal hematocrit (71.4%) and erythrocyte values (91.4%) upon admission, and cats which presented decreased values were equally distributed between SIRS and non-SIRS groups. This may explain the absence of association with SIRS in the multivariate analysis, and confounding factors may have masked the initial statistical significance. Additionally, the fact that an 80% CI was utilized in the univariate analysis does, nonetheless, allow for the inclusion of less significant factors in the analysis and, consequently, some factors may not hold up as strongly when a narrower CI of 95% is applied, as it was in the multivariate analysis.

Some other factors were evaluated in this study, such as certain treatment options and blood tests upon admission. Blood product transfusions and treatment with interferon omega were not considered as risk factors as they did not correlate with SIRS diagnosis. The use of recombinant feline interferon omega in dogs and cats with parvovirosis has been tested, and differing results were observed. A decrease in clinical sign intensity and a more positive outcome were observed in dogs treated with interferon (Martin et al. 2002). In contrast, the same treatment in cats failed to show a positive correlation, although cats had a decreased serologic inflammatory response and a more exacerbated immune reaction (Paltrinieri et al. 2007). In this study, this association was not observed, although it should be taken into account the total number of cats that were treated with interferon (n=3), insufficient for conclusions to be made. Out of these three cats, two were diagnosed with SIRS, one of which died. Although our results failed to show a positive response to interferon treatment, many other factors could have influenced the outcome. Studies including a larger sample should be performed in order

to conclude whether this treatment option is beneficial for cats. As for blood product transfusions, in the initial univariate analysis there was a statistically significant association with SIRS. However, this variable was eliminated from the logistic regression multivariate model due to lack of statistical significance at the chosen significance level or possible confounding factors. Therefore, transfusion of blood products was not considered an independent risk factor for SIRS.

The independent risk factors that were observed in this study were hypoglycemia, hypoalbuminemia and lymphopenia upon admission. It is important to mention that the occurrence of hypoproteinemia was also evaluated and was considered as a potential risk factor during the univariate analysis, but was further eliminated from the multivariate logistic regression model due to its collinearity with albumin values. As such, the presence of hypoproteinemia is also considered as an independent risk factor for SIRS, if substituting hypoalbuminemia in the logistic regression model. These findings regarding association of hypoglycemia, hypoalbuminemia and lymphopenia with a negative outcome are corroborated by other studies. In a study evaluating SIRS and sepsis in dogs after gastrointestinal surgery, hypoalbuminemia and hypoproteinemia were defined as risk factors (Grimes et al. 2011). Hypoalbuminemia and hypoproteinemia are common findings in cats, dogs and humans (Brady et al. 2000; Costello 2015; Klainbart et al. 2017) and have been previously associated with mortality in human patients (Arnau-Barrés et al. 2019; Hu et al. 2021). Whether hypoalbuminemia is effectively a risk factor for SIRS development in this study or a consequence of SIRS onset is still unknown, as the population had underlying infection with FPV which is known to cause hypoalbuminemia (Brady et al. 2000; Costello et al. 2004; Montealegre and Lyons 2021; Sykes and Parrish 2023). It is probable that the presence of hypoalbuminemia upon admission was a direct consequence of FPV, as many patients only fulfilled SIRS criteria during hospitalization. Nonetheless, the results potentially indicate a possible risk factor for onset of SIRS.

Lymphopenia, on the other hand, has been observed as a potential biomarker for immunosuppression in septic patients and has additionally been considered as a prognostic factor for mortality (Hotchkiss, Tinsley, et al. 2001; Gyawali et al. 2019). In the present study, there was a significant statistical association between lymphopenia upon admission and SIRS ($p<0.05$). However, the observed association warrants careful interpretation. While lymphopenia is a common finding in septic cats, it is also a well-documented characteristic of panleukopenia virus infection. Similarly, leukopenia with neutropenia can be observed in both SIRS and panleukopenia virus infections, although leukocytosis is more common than leukopenia in septic cats. The significant association between this variable and SIRS suggests that lymphopenia may indeed serve as an independent risk factor for SIRS. However, it is important to consider the context of FPV infection, as the occurrence of lymphopenia in this

study may be attributed to the viral infection rather than SIRS alone. Further investigation is needed to determine more precisely the predicative power of this factor.

As for glycemia, most septic cats display hypoglycemia, although there are differing results among authors (Hoskins 1993; Brady et al. 2000; Costello 2015). In this study, hypoglycemia upon admission was considered an independent risk factor for SIRS ($p<0.01$). According to these results, a cat which displays hypoglycemia upon admission is 12 times more likely to develop SIRS than a cat which does not. Again, it is unknown whether hypoglycemia predisposes cats to SIRS or whether it is a direct consequence of SIRS or panleukopenia virus infection. In SIRS, hypoglycemia has been attributed to failure of gluconeogenesis following glucose depletion (Van Vught et al. 2016). Moreover, hypoglycemia is a less common finding in cats with parvovirus infections, which makes it more likely that this biochemical finding is in fact related to SIRS. This may explain the results obtained in the statistical analysis that indicate that cats with decreased serum glucose values are in greater risk of developing SIRS. However, we cannot affirm with certainty that these values were not due to an already ongoing septic scenario.

3.4.3. Prognostic Factors for Cats with SIRS

Many attempts to determine prognostic factors for SIRS and sepsis in cats have been made. The identification of prognostic factors in these patients is crucial for the early assessment of severity of the disease and the definition of realistic prognosis, in order to manage expectations and tailor strategies to further optimize patient care.

The variables that were associated with the outcome were maintenance or onset of anorexia or hyporexia during hospitalization ($p<0.001$), maintenance or onset of abnormal mental status during hospitalization ($p<0.00001$), dehydration during hospitalization ($p<0.05$) and lymphopenia upon admission ($p<0.05$). Other variables did not demonstrate a significant association with the outcome and, therefore, were not considered as prognostic factors for septic cats.

Anorexia and hyporexia are common clinical signs among critically ill animals and are of unspecific nature. In the present study, presence of anorexia or hyporexia during hospitalization was considered a negative prognostic factor ($p<0.001$). Although this clinical sign is unspecific, it has been shown to be a common finding in cats with SIRS, and a prolonged inadequate nutritional intake imposes a serious issue and can lead to many complications, such as immunosuppression and secondary organ dysfunction, which are involved in the pathophysiology of SIRS and sepsis (Parsons et al. 2009; Forman 2017; Klainbart et al. 2017). However, it is important to note that 7 out of the 13 cats which maintained anorexia or hyporexia during hospitalization had a feeding tube placed and all but one had negative outcomes. As previously mentioned, parvovirus infection affects nutrient absorption in the

intestines and, therefore, enteric feeding may not effectively address the issue of inadequate nutrient intake. However, one study in dogs showed an increase in appetite and overall wellness in a quicker manner when dogs were subjected to enteric feeding (Mohr et al. 2003). In this study, nonetheless, the maintenance of anorexia and hyporexia mirrors a deeper generalized wellness issue, as even with placement of feeding tubes, cats did not recover their appetite and had a negative outcome.

SIRS and septic patients usually have an altered mental status and this criterium is included in the SOFA and qSOFA score that is currently implemented in human sepsis diagnosis (Brady et al. 2000; Parsons et al. 2009). In this study, all mentation alterations (depressed, stuporous and comatose) were grouped as “abnormal” in order to avoid subjective evaluations that could influence results. The association between maintenance or development of an abnormal mental status during hospitalization and mortality was statistically significant ($p<0.00001$). It has been shown that human septic patients which develop SAE hold a poorer prognosis, and that it may hold prognostic value in septic cats (Osterbur et al. 2014; Gülersoy et al. 2023). This may be due to its pathophysiology, which although is not fully understood, has been shown to involve cerebral hypoxia and tissue lesion caused by inflammatory cytokines. In the present study, all 10 cats that survived were alert during hospitalization, whilst it should be noted that one of the cats which died was comatose upon admission and recovered mentation status throughout hospitalization. Therefore, alertness is not necessarily indicative of a positive prognosis, and status should not be interpreted solely. Nonetheless, these results are in accordance with the human sepsis findings.

Maintenance of dehydration during hospitalization was considered a prognostic factor for mortality in this study ($p<0.05$). Dehydration is defined as an imbalance between fluid intake and loss, and this imbalance may impact physiological processes, particularly in a SIRS or sepsis context. It is also a common finding in cats with FPV and has been shown to increase mortality, which corroborates our findings (Barrs 2019). Although dehydration and hypovolemia are different, one may aggravate the other and, in this case, dehydration may further exacerbate tissue hypoperfusion, leading to tissue hypoxia and organ dysfunction, already prominent in septic environments. In this study, all cats were subjected to fluid therapy with Ringer’s Lactate, although the fluid rates were not evaluated as they were calculated and attributed by the on-call veterinarians, which inherently imposes subjective decisions. Nonetheless, patients who developed or maintained dehydration levels during hospitalization were receiving fluids intravenously, which may possibly indicate inability to respond to fluid therapy. This, in turn, may imply hypovolemia and, eventually, increased risk of developing septic shock. Unfortunately, the present study did not take into consideration systolic arterial pressures, vasopressor administration and serum lactate values, as for most of the included patients that information was not available or those interventions were not performed.

Therefore, it is not possible to determine which patients were hypotensive or with hyperlactatemia. Pulse measurement, however, was accounted for and was not statistically associated with the outcome (p<0.2). On the other hand, dehydration may, nonetheless, have detrimental effects on other organ systems, such as the renal and cardiovascular systems. Depleted fluid levels can strain renal function and further increase mortality risk, especially in cats. Although creatinine values were normal for all patients upon admission and therefore were not associated with the outcome (p=1), consistent measures of creatinine along the hospitalization period would permit a broader view of possible SIRS-induced renal dysfunction in this population. Additionally, serum creatinine has been considered an insensitive biomarker for early renal dysfunction, and urinary clearance or SDMA values would be preferred. Conclusively, the fact that continued dehydration was significantly associated with mortality should be interpreted with caution, considering the complex interplay between fluid balance, tissue hypoperfusion and organ dysfunction, as well as the subjectivity behind the hydration level evaluation. Nonetheless, these results may provide insights into the importance of adequate fluid therapy and the risk behind inadequate fluid responsiveness in a SIRS context. Further studies evaluating glomerular filtration rates, arterial systolic pressures and serum lactate values would enhance our comprehension.

Lymphopenia showed an association with mortality (p<0.05), concluding that cats with SIRS that presented lymphopenia upon admission were approximately 12 times more likely to have a negative prognosis than cats with increased or normal lymphocyte counts. As mentioned previously, lymphopenia is a common finding in septic and cats with systemic inflammatory response syndrome and may be due to the onset of immunosuppression and lymphocyte exhaustion and apoptosis (Hotchkiss, Tinsley, et al. 2001; Greiner et al. 2008; Berlot and Passero 2016; Klainbart et al. 2017). However, lymphopenia is also a common finding in cats with parvovirus infection and presence of low lymphocyte values may be a cause of the underlying parvovirus infection which characterizes the study population (Sykes and Parrish 2023). Nonetheless, the statistically significant association which has been made with mortality should not be overseen in this context, as these results are in agreement with recent findings in human sepsis.

As for all the remaining variables, no statistical association with the outcome was observed (Table 8). In fact, it is possible that the absence of association with the outcome is because of the small study population. In this study, neither hypothermia nor fever upon admission were correlated with the outcome, which supports the current literature (Scotti et al. 2019), as well as hemogram alterations, which is contrary to some studies in septic cats. However, toxic neutrophils, which have been mentioned to have some predicative value, were not considered in this study, as for many cats this information was unavailable. Hypoalbuminemia was also discarded as a potential prognostic factor for mortality, which is in

agreement with current reports in cats (King 1994; DeClue et al. 2011) but contradicts results in human septic patients (Arnau-Barrés et al. 2019; Cakir and Turan 2021; Hu et al. 2021). It is likely that these results were influenced by the small sample size and the underlying disease which itself is known to cause hypoalbuminemia. It is also possible that a few patients were admitted to the ICU before a significant decrease in serum albumin values. Concerning this hypothesis, albumin values should ideally be quantified daily or at least throughout hospitalization, to evaluate the albumin evolution and determine whether it is rising or descending. Other than this, further studies on SIRS and septic cats with different causes should be performed, in order to obtain an unbiased conclusion.

It would be expected for the presence of diarrhea or blood in the stool to be associated with the outcome. This is because, as mentioned previously, FPV replicates itself in the intestinal mucosa, promoting lesions to the intestinal mucosal barrier and bacterial translocation from the gut to the bloodstream. As such, it would be expected that more aggressive gastrointestinal signs, such as vomiting and diarrhea, would be associated with poorer outcomes, especially in cases with hematochezia which could cause further blood loss. Moreover, presence of diarrhea and vomiting can aggravate dehydration and malabsorption, both factors that may lead to a more fragilized state. However, this was not observed in the present study (p<0.2).

Treatment was also evaluated in the present study. All cats were treated with intravenous fluid therapy with Ringer's Lactate and Amoxicilin with clavulanic acid. Doses and timings were not accounted for, as this information was not available. Remaining antibiotics were evaluated and correlated with the outcome, with no results. Only 14 cats were treated with marbofloxacin, and of these, 9 died. Most cats (80%) were treated with metronidazole, although it did not influence the outcome. According to recent literature, administration of appropriate empirical antibiotics in septic cats upon admission is associated with a positive outcome (Scotti et al. 2019). In the present study, this association was not made, although it is not possible to determine at what time or day the antibiotics were administered. Nonetheless, most cats were treated with empirical antibiotics according to the recent guidelines/suggestions. Further studies comparing different antibiotic associations as well as timings and doses should be performed to better comprehend the significance behind the current guidelines.

3.5. Limitations

Limitations of this study included those inherent to retrospective investigations, such as incomplete medical records, subjective evaluations and differing opinions, inconsistent reports of physical examinations and analytical results and differences in in-hospital care and management. Moreover, due to the limited and small number of SIRS patients, a multivariate analysis was not possible in the evaluation of prognostic factors.

3.6. Conclusion

The present study aimed to investigate potential risk factors for SIRS in cats, as well as prognostic factors, based on clinical signs and basic laboratory findings. Our goal was indeed to help define factors that could aid clinicians in early SIRS detection, to further adapt treatment approaches and individual management, as well as identify prognostic factors that would accelerate the determination of favorable or unfavorable outcomes in a cost-effective and timely manner.

Both 1991 and 2001 SIRS criteria demonstrated a significant statistical association with the outcome, when considering only cats with two abnormal physical exams in a 24-hour period, as opposed to one single physical exam. This procedure is adequate to increase specificity in sepsis diagnosis and may, therefore, be applied in a clinical scenario. However, it is important to acknowledge the evolution in sepsis definitions in human medicine as opposed to the absence of such consensus in the veterinary community, in the attempts to adapt current definitions and new criteria to animal patients.

The independent risk factors that were identified in the present study were hypoalbuminemia, hypoproteinemia, hypoglycemia and lymphopenia upon admission. Although it was not determined whether these were caused by SIRS or direct risk factors for SIRS development, a better understanding of these common findings in septic cats was established and may aid towards a more comprehensive insight of sepsis in cats. Although weight was considered as non-independent in the multivariate analysis, the results in the CI demonstrated a considerable level of imprecision. Therefore, these results showed the importance of future investigations with a larger and a more inclusive database to determine the associations between weight, obesity and SIRS in cats.

As for prognostic factors, anorexia or hyporexia, dehydration and abnormal mental status during hospitalization, as well as lymphopenia upon admission were found to have a significant statistical association with the outcome. Although most of these factors are unspecific, these results highlighted the varied and heterogenous clinical presentations and individual inflammatory responses among cats with SIRS, and the need for more studies evaluating predicative indicators in this population, as well as the necessity to refine prognostic models for septic cats. Ultimately, the use of different antibiotics proved not to affect outcome, although the studied population was small and the treatment protocols did not differ significantly among patients. As such, studies including larger study samples and different treatment protocols should be performed, with emphasis on antibiotic administration times. Additionally, prospective studies evaluating biomarkers for sepsis in cats are recommended, as the in-depth characterization of these factors will ultimately lead to a better understanding of SIRS and sepsis in feline patients, and contribute to develop better management strategies and treatment approaches.

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Appendixes

Appendix 1 – Mean, Median, Standard Deviation and descriptive statistics of Age for all 70 cats with Feline Panleukopenia Virus Infection

Descriptive Statistics	
Mean	1,48571048
Standard Error	0,26758894
Median	0,58333333
Mode	0,5
Standard Deviation	2,23880973
Sample Variance	5,01226903
Kurtosis	14,3974202
Skewness	3,37260746
Range	13,8333333
Minimum	0,16666667
Maximum	14
Sum	103,999733
Count	70

Appendix 2 – Shapiro-Wilk Normality Test of "Age" in all 70 cats with Feline Panleukopenia Virus Infection

W= 0.58295	p-value= 8.485^{-13}

Appendix 3 – Mean, Median, Standard Deviation and descriptive statistics of Age for SIRS positive cats

Descriptive Statistics	
Mean	1,27668133
Standard Error	0,36377212
Median	0,5
Mode	0,5
Standard Deviation	1,81886058
Sample Variance	3,30825382
Kurtosis	2,54747845
Skewness	1,94496956
Range	5,83333333
Minimum	0,16666667
Maximum	6
Sum	31,9170333
Count	25

Appendix 4 - Shapiro-Wilk Normality Test of "Age" in 25 SIRS positive cats

W= 0.62021	p-value= 0.0000007283

Appendix 5 – Abstract submitted to the 48th World Small Animal Veterinary Association (WSAVA) World Congress – "SIRS in Feline Patients – Identification of Risk and Prognostic Factors using Viral Panleukopenia as a model"

SIRS in Feline Patients – Identification of Risk and Prognostic Factors using Viral Panleukopenia as a model

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Abstract

Introduction. Sepsis and Systemic Inflammatory Response Syndrome (SIRS) are serious medical conditions responsible for many deaths among animals. Understanding potential risk factors for SIRS in feline patients, as well as prognostic factors is crucial for effective management and prompt identification of sepsis.

Objectives. To investigate risk factors for SIRS and prognostic factors in cats, using panleukopenia as a model.

Methods. Seventy panleukopenia-positive cats were analyzed retrospectively. All cats underwent the 1991 and 2001 SIRS criteria. Fisher's exact test was performed to assess associations with the outcome. Univariate analysis and multivariate logistic regression analysis followed, to evaluate potential independent risk factors for SIRS. The focus was then narrowed to 25 SIRS positive cats and univariate analysis was performed to determine whether any variables were associated with the outcome.

Results. Both SIRS criteria may be useful in a clinical setting, if there are two abnormal physical exams within a 24-hour period. Hypoalbuminemia, hypoglycemia, and lymphopenia were found to be crucial risk factors for SIRS ($p<0.05$). Lymphopenia, maintenance or onset of anorexia/hyporexia, dehydration and abnormal mental status during hospitalization can be considered negative prognostic factors ($p<0.05$).

Conclusion. Our study contributes to a better understanding and clinical management of feline SIRS, enhancing the ability to identify cats at-risk and improving prognostic assessments for tailored and effective treatment interventions and management approaches.

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