

Surviving Sepsis in Veterinary Medicine

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Introduction

Sepsis and septic shock are major causes for morbidity and mortality in patients in both human and veterinary medicine. In people, the true incidence of sepsis is unknown since there is variability in the reported cases. A recent report shows that clinical data from 409 hospitals, sepsis was present in 6% of adult hospitalizations, and in contrast to claims-based analyses, neither the incidence of sepsis nor the combined outcome of death or discharge to hospice changed significantly between 2009-2014.¹ Regardless of the precision of the data, it is clear that the burden in health and health care expenses due to direct and indirect costs in treating patients are significant.

Published data indicate that sepsis leads the top 5 most expensive conditions treated in U.S. hospitals.² In Veterinary Medicine, the incidence and the burden of disease is poorly described. As in people, sepsis in Veterinary Medicine is likely to be a major cause of mortality in hospitalized dogs and cats. In fact, available data shows that mortality can reach 50%.³ Sepsis is a complex syndrome that its definitions change on a regular basis, and a literature search for the last 5 years yielded near 9,000 citations covering a wide of areas including pathogenesis, biomarkers, clinical trials, etc.

Our purpose is to provide a summary to primary care Veterinarians of the most important aspects in: a) Mechanisms and Pathogenesis, b) Laboratory diagnosis focusing on Biomarkers, and c) Diagnosis and Treatment Challenges in Small Animals. These sections will assist Veterinarians in the appropriate categorization of a patient as having systemic inflammatory response, sepsis or septic shock and should bring the attention of all personnel involved to rapidly identify and manage the syndrome and organ dysfunctions and hence, an unfavorable outcome. Also, it will help for preparedness on resource allocation for the best possible patient's care.

Chapter 1. Mechanisms and Pathogenesis.

Definitions.^{4,5}

What is Systemic Inflammatory Response?

- Clinical manifestation of systemic inflammation, which results from either:
 - Infectious insult (septic SIRS).
 - Noninfectious insult (non-septic or sterile SIRS).

What is Sepsis?

- Dysregulated (pro & anti) inflammatory response to infection/trauma.
- Progressive, organic slow and causing multiple vital organ failure coupled with critical reduction in tissue perfusion.

What is septic shock?

- A subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality.
- Despite adequate intravascular fluid resuscitation, sepsis-associated:
 - Acute circulatory failure.
 - Persistent arterial hypotension.

What is multi-organ dysfunction syndrome (MODS)?

- Physiologic derangements of at least 2 major organ systems associated with SIRS.
- In people there is the Sequential Organ Failure Assessment (SOFA) score (0 to 4): Respiratory, Coagulation, Liver, Cardiovascular, Central Nervous System, and Renal.⁶

What is CARS?

- The counter-inflammatory response syndrome.
- Anti-inflammatory cytokines IL-4 and IL-10 that are responsible for decreasing the pro-inflammatory effect of TNF- α , IL-1, IL-6, and IL-8.

What is MARS?

- Is a mixed antagonist response syndrome.
- Triggered between SIRS (Hyper-inflammatory) and CARS (hypo-inflammatory).

Pathophysiology of sepsis (See Table below)

Table 1. Outline of events leading to SIRS, ARS, MARS, Sepsis, Septic Shock and MODS.⁷

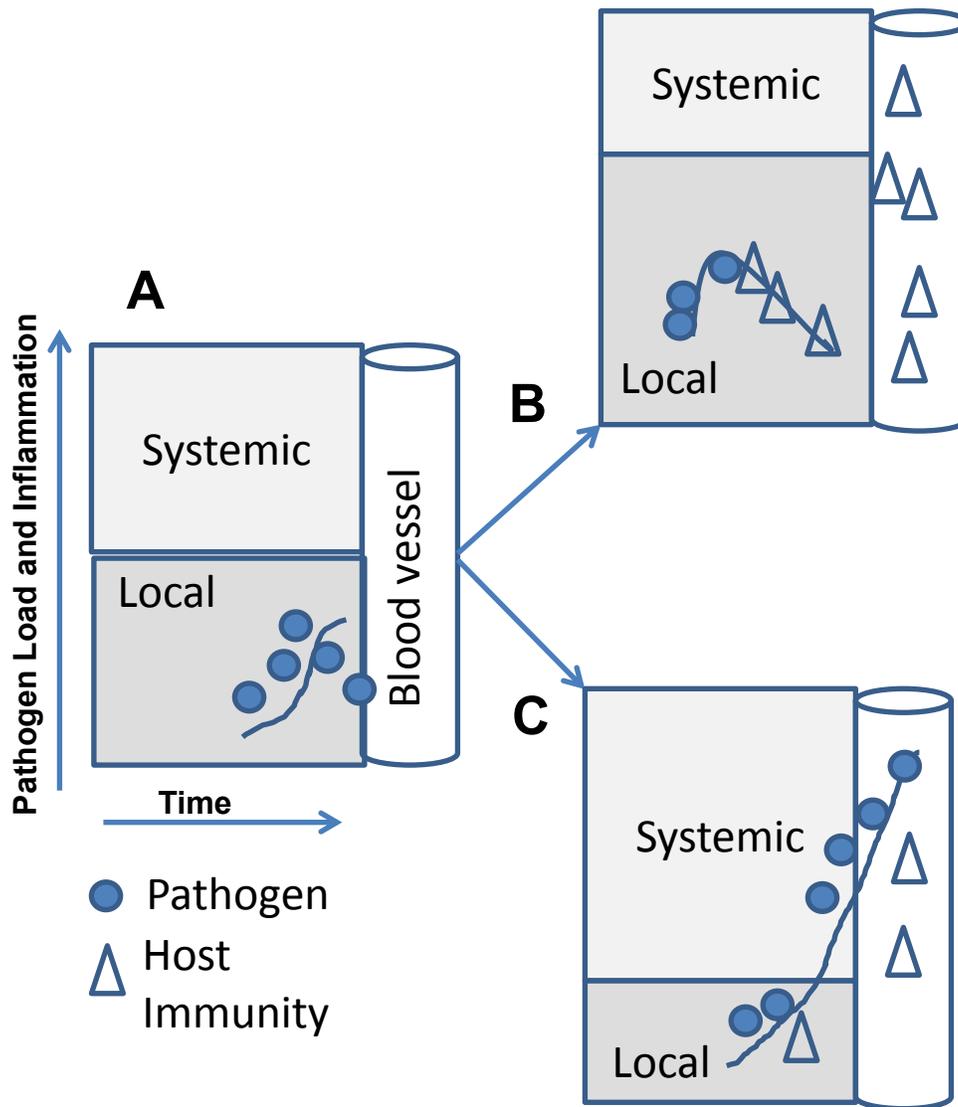
1. Insult	Uncontrolled infection, major trauma, circulatory shock, tissue necrosis, apoptosis, anaphylaxis
↓	↓
2. Triggers	Receptors: Pathogen-associated molecular patterns (PAMPs) Damage-associated molecular patterns (DAMPs)
↓	↓
3. Sensors and effector cells	Complex protein systems (coagulation), vascular and tissue cells and blood and lymphatic cells (neutrophils, platelets)
↓	↓
4. Mediators and biomarkers	>300 cytokines/The Cytokine Storm, lactic acid, coagulation proteins, me
↓	↓
5. Impact on organ function	Brain: confusion, Lung: respiratory distress, Cardiovascular: shock, liver: failure Kidney: failure, Gut – loss of barrier, microcirculation: edema, capillary leak, DIC.
↓	↓
6. Outcome	Effective source control: Normalization and resolution Ineffective source controls: MOS, death

Immune response: local vs systemic:

Concept: The immune response to pathogens triggers local reactions that with time, become systemic. Locally beneficial host defense mechanisms can become detrimental when activated systemically. The septic reaction travels via the vascular system to spread inflammation throughout the body leading to organ failure. Early infections represent a race between the ability of pathogens to multiply and spread and the hosts' ability to sequester and kill pathogens before they disseminate. This race starts after resident innate immune cells expressing TLRs recognize pathogens, leading to local vasodilation, increased vascular permeability, recruitment of neutrophils and monocytes, and local coagulopathy. If the inoculum is high, the pathogen evades host defenses, or if the host response is slow to gain control over multiplying pathogens, then both the pathogen and the inflammatory response aimed at pathogen containment extend beyond the local

environment, and both systemic infection and systemic inflammation. Figure 1 summarizes how sepsis is a numeric and geographic race between bacterial growth and host defense.

Figure 1. Local to systemic inflammatory response.



In the Figure 1, the following steps can be recognized : A) after the initial inoculum, bacteria or other pathogens begin to propagate within local compartments, B) if the immune response is sufficiently fast, then the spread of pathogens is limited by defense mechanisms and C) if the infection spread outside a single compartment and the infection point where specific host defense mechanisms shift from benefit to detriment is crossed, then both infection and the inflammatory response to the infection become systemic, resulting in diffuse organ injury and shock. This is an ineffective immune response with systemic pathogen dissemination, systemically elevated cytokines (The Cytokine Storm), and multiple organ failure.

What are the TOLL receptors and why they are important?

Immune response is triggered by receptor sensing either bacterial products or damaged cell-derived products. Both receptors will initiate the pro inflammatory response as a mechanism of defense. The cells of immune system express various pattern recognition receptors (PRRs) that detect danger via

recognizing specific pathogen-associated molecular patterns (PAMPs) and mount a specific immune response. There are 13 TLRs described.⁸

Table 1. A list of the TLR receptors and their pathogen ligands.⁹

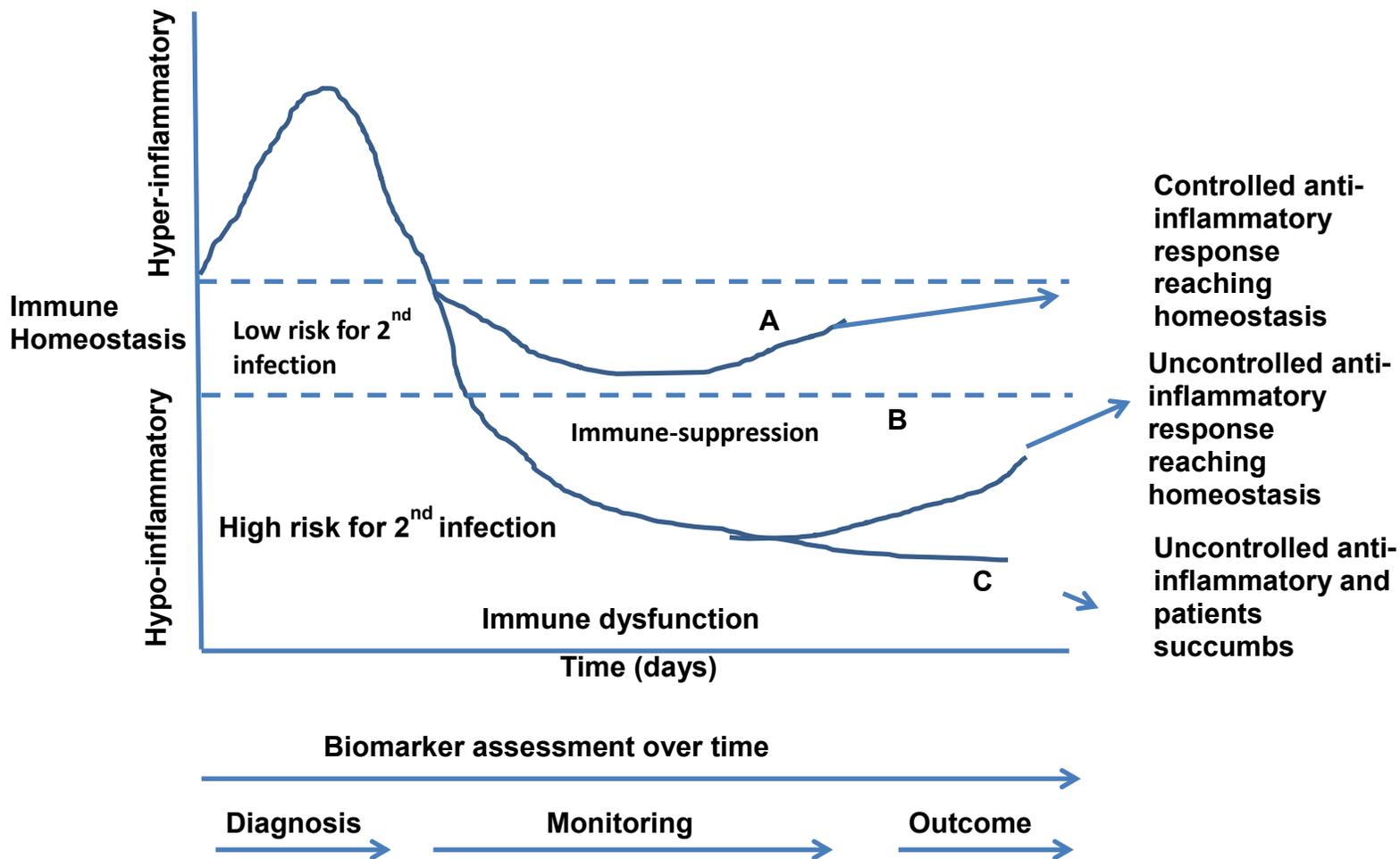
PAMPs	TOLL (TLR)	Pathogen
Zymozan,	1,2,6	Gram positives
Endotoxins	4 (most important)	Gran negatives
Flagelin	5	Bacteria and e flagella
dsRNA	3	Virus
dsDNA	7, 8	Virus
CpG	9	Bacteria, DNA

Cells are activated and the inflammasomes are triggered. An inflammasome is a macromolecular complex that is required for caspase-1 activation and cleavage of inactive pro-IL-1 to its biologically active form IL-1 and also IL18. IL-1 is an endogenous fever trigger.¹⁰ Activation of inflammasomes during sepsis and trauma serve to amplify inflammatory signaling influencing the Cytokine Storm. Nine types have been identified.¹¹

Sepsis: bifasic response: hiperinflammatory and hipoinflammatory.¹²

Immune response during SIRS, Sepsis and Septic Shock has 2 phases: hyper-inflammatory and a hypo-inflammatory which lead to three possible outcomes: a) controlled anti-inflammatory response reaching homeostasis, b) uncontrolled anti-inflammatory response reaching homeostasis and c) uncontrolled anti-inflammatory and patient dies. When diagnosing and treating patients, SIRS, Sepsis, Septic Shock and MODS we need to consider that there is a temporal relationship during the course of the disease. Therefore, monitoring strategies and biomarkers will change over time. During the hyper inflammatory phase, there is a low risk for a second bacterial infection, In contrast, during the hypo inflammatory phase, there is a high risk for a secondary pathogen infection, immunosuppression and immune dysfunction. Biomarker evaluations can be performed over time, as well as monitoring therapy. The next figure depicts the disease evolution over time.

Figure 2. The immune response in sepsis.^{13,14}



Neutrophils in sepsis and organ failure

Review.

- First line of defense.
- Main cell type involved in acute inflammation
- Main functions: secretion of molecules, migration and phagocytosis.
- Dysfunctional neutrophil biology in sepsis will lead to significant changes that will contribute to the development of secondary complications and MOD.
- When infection occurs: mobilization, marginalization and rolling in the endothelium, and adhesion and transmigration through the wall of the blood vessels with significant changes in their morphology and biology.

Changes in the neutrophil biology during sepsis:

- Changes in the neutrophils elasticity.¹⁵
 - Pro-inflammatory mediators and released bacterial products result increased leukocyte stiffness.
 - Neutrophils become sequestered in the capillary beds (lungs, liver).

- Reduction of leukocyte movements during sepsis may contribute even more to neutrophil sequestration and vascular occlusion, thus promoting tissue ischemia and dysfunction of various organs.
- Alterations in neutrophil-endothelial cell and chemotaxis.¹⁵
 - Profiles of adhesion molecule expression in neutrophils, endothelial cells further promote firm and neutrophil adhesion in the vasculature.
 - Movement to the sites of infection controlled by cytokines and microparticles
- Deterioration of neutrophil migration ¹⁶
 - Compromised due to excessive release of pro-inflammatory mediators

What are microparticles?

- Microparticles are proinflammatory vesicles and procoagulants released from neutrophils, platelets and other cells.¹⁷
- Activation of resting platelets, increased increase P-selectin expression, and perpetuate thrombus formation.¹⁸
- Trigger proinflammatory mediators by activating endothelial cells (IL-6).¹⁸
- Activation of classic pathway of complement and fix C4 and C3 fragments.¹⁸

Expansion of inflammation by neutrophils.¹⁹

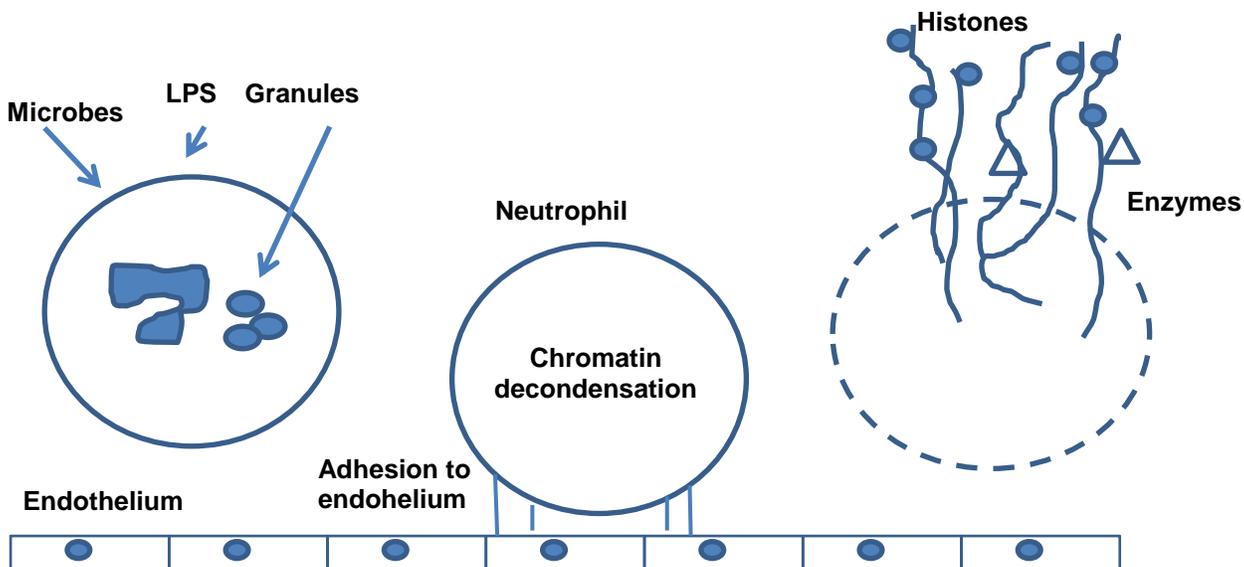
- Acute phase, neutrophils entering the circulation can spread inflammation in other organs, and leading to damage.
- Late phases, there is a state of immune refractoriness with reasonably high amounts of anti-inflammatory cytokines and specific inhibitors of cytokines.
- Lack of maintenance of the balance between excessive inflammation vs anti-inflammatory mechanisms.
- Systemic neutrophil activation induce the release of NETs into the blood vessels causing endothelial damage, culminating with worsening sepsis and possible death.

What are NETs?

Are neutrophil's weapons against bacteria. To kill these pathogens neutrophils use strategies such as phagocytosis, degranulation and NETs formation. The latter, are webs of DNA coated in anti-microbial proteins that are released into the vasculature during sepsis and contribute to organ damage.

Once the neutrophils are activated: There is release of nucleosomes in response to stimuli by infection or inflammation. The neutrophil extracellular traps (NETs) are composed of nucleosomes with granular components . Networks link and kill microbes. The nets also immobilize platelets and erythrocytes triggering DIC.²⁰ Figure 3 summarizes the NETs formation.

Figure 3. Neutrophil activation and NET formation.²¹



Platelets in sepsis and organ failure.

Review.²²

- Small (2–4 μm).
- Anucleate.
- Discoid-shaped cytoplasmic fragments released in the bloodstream during the fragmentation of polyploid megakaryocytes in bone marrow sinusoidal blood vessels.
- Short lifespan, of up to 10 days.
- Rapidly accumulate at the site of infection.
- Express Toll-like receptors.

Platelet tasks²³

- Hemostasis
 - Detection of vascular breach
 - Response to endothelial alarms
 - Clot formation
 - Regulation of vessel permeability
 - Sealing of vascular breaches during leukocyte transmigration
 - Blood-lymph separation
- Immunity
 - Direct pathogen detection
 - Pathogen binding and degranulation
 - Leukocyte recruitment
 - Response to leukocyte alarms (NETs)
 - Physical interaction with leukocytes signal exchange

Antimicrobial mechanisms in platelets.²⁴

- Activated metabolic status.
- Expression of receptors mediating increased adhesion to injured or infected tissues.
- Motility toward and intensification at sites of tissue injury or infection mediated by C3a and C5a.
- Generation of reactive oxygen species including superoxide, peroxide, and hydroxyl radicals.
- Extension of pseudopodia that interact with microbial pathogens and host cells.
- Cytoskeletal remodeling to facilitate granule mobilization and organization.
- Degranulation and processing of preformed granule molecules, including host defense peptides.
- Platelets bridge innate and adaptive immunity.

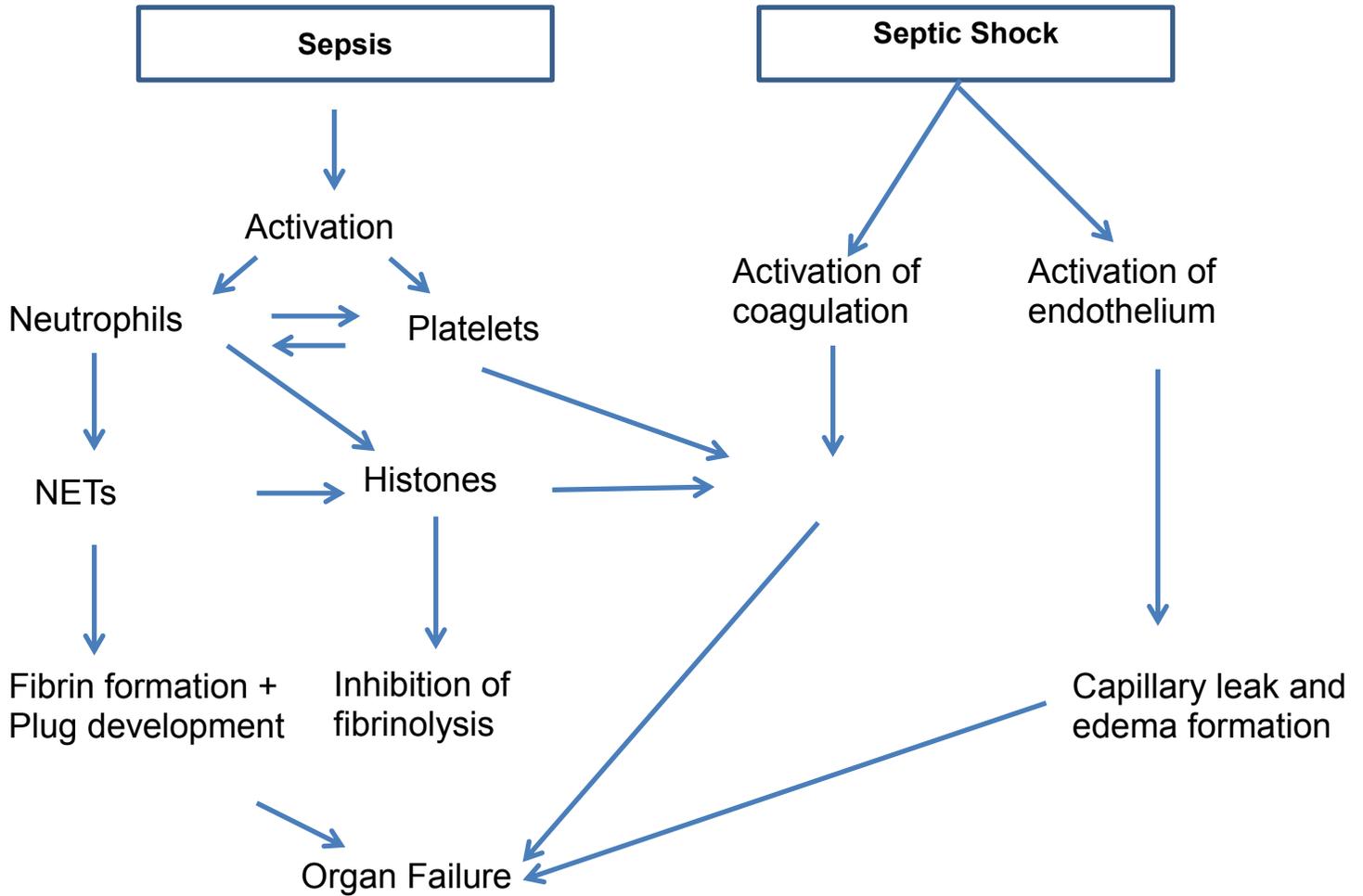
Platelet microparticles.²⁵

- Is a distributed storage pool of bioactive effectors, exerting pro-inflammatory and pro-thrombotic properties in the immediate microenvironment of their formation.
- Contribute to myocardial dysfunction in sepsis by decreasing myocardial.

How platelets cause multiple organ failure?

- Immune cell recruitment and hyper inflammation.²⁴
- Development of vaso-occlusive thrombi in capillary vascular beds.²⁴
- Direct cell toxic effects of platelets and platelet derived micro particles on endothelial cells.²⁴
- Formation of DIC.²⁴
- Platelet-endothelial adhesion, platelet-leukocyte aggregates, and NETs all contribute to the formation of micro-thrombi in small vessels.²⁶
- The cells involved release cytokines and chemokines resulting cellular recruitment, which can become pathological self-sustaining dysregulated process resulting in septic shock.²⁵
- During sepsis, even when organ perfusion is preserved, patchy areas of reduced oxygen delivery with consequent mitochondrial dysfunction influencing mortality.²⁵
- Platelets will induce.²⁵
 - Acute respiratory distress (ARD).
 - Disseminated Intravascular Coagulation (DIC).
 - Acute Kidney Injury (AKI).

Figure 4. Summary the consequences of neutrophil-platelet-endothelium activation and interplay in sepsis/septic in shock and organ failure.²¹



The Cytokine Storm

What is the cytokine storm?

- Is the systemic expression of a healthy and vigorous immune system resulting in the release of more than 150 known inflammatory mediators.
- Consequences:
 - Local and systemic inflammation.

What are the cytokines most commonly associated with the storm?²⁷

- See Table 2. Cytokines associated with the storm.

Table 2. Cytokines associated with the storm.

Type	Actions
Interferons	Regulation innate immunity
Interleukins (pro-inflammatory IL-1 α and IL-1 β ; antflammatory IL-10)	Growth and differentiation (pro-inflammatory)
Chemokines	Leukocyte recruitment (pro-inflammatory)
Colony-stimulating factors	Stimulation of progenitor cells
Tumor necrosis factor	Prinflammatory

- Cytokine storm begins at a local site and spreads throughout the body via the systemic circulation expanding to other organs.
- Acute lung injury progresses into respiratory distress.
- Hypotension, hyper- or hypothermia, leukocytosis or leukopenia, and often thrombocytopenia.

Surviving sepsis in Veterinary Medicine: Chapter 2- Laboratory diagnosis

Introduction:

The septic response is an extremely complex chain of events involving inflammatory and anti-inflammatory processes, humoral and cellular reactions and circulatory abnormalities.

An early diagnosis of sepsis helps to initiate rapid treatment, improve outcomes and reduce unnecessary antibiotic therapy. Diagnostic biomolecular markers can aid veterinarians to simplify, accelerate and objectify outcomes. Process from diagnosis and process monitoring to verification and timely correction of therapy.

Currently, there is no ideal and clinical gold standard for the diagnosis of sepsis, as microbiology may not be sensitive enough and laboratory tests are unspecific for use as a reference standard.

What are biomarkers?²⁸

- Characteristic that is objectively measured and evaluated as an indicator of normal biological process, pathogenic process, or pharmacologic response to a therapeutic intervention.
- Usefulness is evaluated by:
 - Capacity to provide timely information beyond what is readily available from routine physiologic and clinical data (Speed + Accuracy).

- Sensitivity and specificity must be an acceptable value.

Classification of sepsis markers

- Cytokine/chemokine biomarkers - IL-1 receptor antagonist & TNF.
- Cell markers – CD40.
- Receptor - Fc-gamma RIII.
- Coagulation - Activated partial thromboplastin time (aPTT).
- Vascular endothelial damage - Platelet-derived growth factor (PDGF), ELAM1, ICAM1,
- Vasodilation - Angiotensin converting enzyme (ACE).
- Organ dysfunction – Troponin.
- **Acute phase proteins – Calcitonin, Hecpidin.**
- **Metabolic – Lactate.**
- Vasodilation - Neuropeptide, NO, Substance P.

Roles of the Biomarkers²⁹.

- Identify patient with ↑ probability of disease, adverse outcome, or benefit from intervention
- Identify presence or absence of pathologic state or process
- Aid in risk stratification/prognosis
- Monitor response to an intervention or treatment
- Serve as surrogate endpoint

Lactate

What is lactate?

- Lactate is a by-product of glycolysis, energy pack.
- Glycolysis has 2 enzymatic pathways:
 - Cytoplasm – first set of enzymatic reactions - mostly anaerobic – poorly perfused tissues - 1 glucose will produce 2 pyruvates and 2 ATP, pyruvate either is converted into lactate or moves to the mitochondria into Krebs cycle for the second set of reactions.
 - Mitochondria – second set of reactions - mostly aerobic- lactate is oxidized and produces 18 ATPs. If low oxygen exists, pyruvate is converted into lactate to maintain energy. Lactate/pyruvate ration increases (normal pyruvate/lactate ratio is 10 pyruvate:1 lactate). Once molecular oxygen is again available, and if the mitochondrial function is preserved, the excess lactate is rapidly metabolized back through pyruvate into CO₂ and H₂O via the Krebs cycle.

Where the lactate is metabolized?

- Liver.
- Kidney – lactate clearance – pH and blood flow critical for clearance .

Lactate as a marker for cellular stress.

- Under stress, lactate is a source of energy in the same cell where it is produced and also in other cells where it can be used as an important fuel for oxidation and glucose generation.
- Lactate Clearance as independent predictor of mortality.
- Targeting resuscitation in sepsis to achieve a lactate 'clearance' of at least 10%.

Sampling techniques

- Lactate production by red blood cells continues following blood sampling, blood samples must be analyzed rapidly (<5 minutes) following sampling.³⁰
- Blood samples submitted for laboratory analysis should be collected in tubes containing sodium fluoride (NaF).²⁸
- Solutions containing 5% dextrose, in normal saline or Ringer's lactate, have been shown to result in significant increases in blood lactate concentration over time.³¹
- Stress and struggling (induced by bathing) can increase a tenfold in plasma lactate concentrations in cats.²⁹
- Regional differences: minimal, venous samples – locally concentrations, arterial – systemic levels.³²

Laboratory methods for serial lactate measurements.

- Central laboratory - ~ 30 min depending on patient load and sample management.
 - Enzymatic.
- Blood gas analyzer - ~30 min depending on patient load and sample management.
 - Biosensor.
- Handheld devices (PoC) – 15 seconds.
 - Enzymatic biosensors.

Early lactate measurements

- To allow a correct triage and address the patient to the proper hospital structure.
- To allow the proper therapy to be administered with no delay (Time-to-antibiotics, dedicated therapy).
- To allow an efficient patients' monitoring @ ward.
- Delays – will impact patient outcome and cost.
- Reduced TAT (Turn-Around-Time).
- Reduced Sample volume.
- Improved key performance indicators.
 - TTT (Time-to-Triage) or TTA (Time-to-Antibiotics).

Lactate concentrations.³³

Table 2. Lactate Concentrations in Mature Dogs and Cats.

Lactate concentration (mmol/L)	Significance
<2.5	Normal
2.5-4.9	Mild elevation
5-7	Moderate elevation
>7	Severe elevation

Interpretation of lactate concentrations.³⁴

- Increased blood lactate can only be caused by increased anaerobic or aerobic lactate production, and eventually combined with decreased lactate clearance.
- Aerobic lactate production, either global or focal (especially in the lungs), is the result of activation of the inflammation cascade.

Figure 5. Interpretation of hyperlactemia.

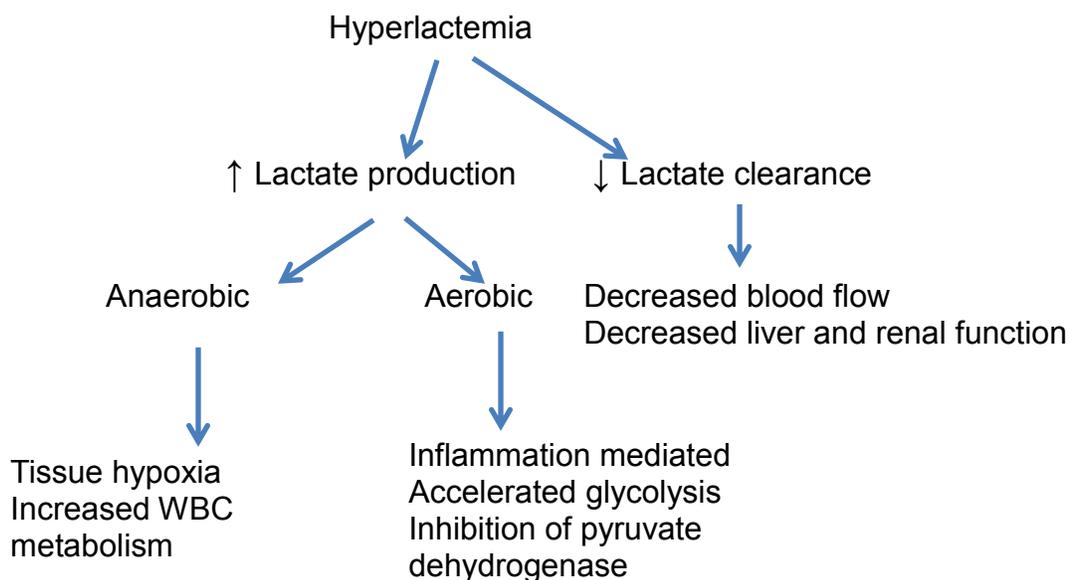


Figure 5 illustrates the blood lactate concentrations reflecting the balance between lactate production, either anaerobic (mainly in tissue hypoxia) or aerobic, and lactate clearance. The clearance is the sum of the endogenous oxidative-phosphorylation lactate production and the

additional lactate production under the influence of inflammation, and lactate clearance, mainly by the liver.

Hyperlactemia.³⁵

Table 3 details the types of hyper-lactemia.

Type A Hyper-lactemia (due to tissue hypoxia)		
Increased Oxygen demand	Decreased oxygen delivery	
Exercise Seizures Shivering	<u>Septic shock</u> Blood loss Severe anemia	
Type B Hyper-lactemia (not due to hypoxia)		
B ₁ Associated with underlying disease	B ₂ Associated with Drugs or Toxins	B ₃ Inborn errors in Metabolism
<u>Sepsis</u> Diabetes Liver Disease Neoplasia	Acetaminophen Activated Charcoal Cyanide Ethylene Glycol Methanol	Mitochondrial myopathies Enzymatic diseases

Clinical use of lactate in critical care patients

- In people.
 - Lactate monitored pts had significantly less length of stay, and decreased mortality and early lactate detection and serial measurements provide clinical benefit.³⁶
 - Lactate clearance was higher in survivors than in non-survivors, and low clearance is predictor of death at day 28.³⁷
- In Veterinary Medicine.
 - Lactate concentrations and lactate clearance were good prognostic indicators in dogs with septic peritonitis.³⁸
 - Point-of-care rapid devices are useful in the clinic.³⁹

Procalcitonin

What is procalcitonin (PCT)?⁴⁰

- Procalcitonin is a 116 amino-acid peptide - Precursor of the hormone Calcitonin.
- Synthesis in healthy persons in the C-Cells of the thyroid.
- Endocrine regulator.
- PCT is enzymatically converted to calcitonin and then stored in endocrine granules.

- Released only under certain stress (e.g. magnesium, gastrin).

What is the role of PCT in the absence of infection?⁴¹

- Alternative (cytokine-like) pathway during sepsis: 'Hormokine'.
- Bacterial toxins (gran +/gram-) and cytokines stimulate production of Procalcitonin in all parenchymal tissue.
- PCT is immediately released into the bloodstream is short lived.

Why Procalcitonin can be used as a biomarker in sepsis?⁴²

- PCT mRNA is expressed in many tissues including lung, liver, kidney, adrenal, colon, leukocytes.

Methods to detect Procalcitonin.⁴³

- Enzymatic Immunoassays.
- Chemiluminescent.
- Immunofluorescent: time-resolved amplified cryptate emission (TRACE).
 - Based on non-radiating energy transfer from donor molecule (europium cryptate) to acceptor molecule (XL665) as a result of the completed immune reaction.

Procalcitonin improves diagnosis in people.⁴⁴

- PCT levels accurately differentiate sepsis from noninfectious inflammation*
- PCT has been demonstrated to be the best marker for differentiating patients with sepsis from those with systemic inflammatory reaction not related to infectious cause.

Clinically relevant levels in antibiotic therapy in people⁴⁵ Importance is to manage antibiotic therapy.

Table 4. Procalcitonin levels and antibiotic therapy in sepsis.

Procalcitonin level	Interpretation	Antibiotic therapy
<0.1 µ/L	Bacterial infection very unlikely	Withhold
0.1-0.25 µ/L	Bacterial infection unlikely	Withhold
0.25-0.5 µ/L	Bacterial infection likely	Start or continue
>0.5 µ/L	Bacterial infection very likely	Start or continue

Procalcitonin in Veterinary Medicine.

- Detection of dog Procalcitonin
 - No very consistent.⁴⁶

- SIRS detection
 - PCT quantified by ELISA is helpful the detection SIRS in horses⁴⁷.
- Detection – lung diseases.
 - PCT is useful as a biomarker in equine lung diseases.⁴⁸
- Organ dysfunction
 - Serial procalcitonin monitoring may offer valuable prognostic information in canine sepsis, wherein early decreases in PCT concentrations are associated with survival.⁴⁹

Conclusions.

Lactate

- Useful marker for organ dysfunction.
- Endpoint for resuscitation in patients with sepsis and septic shock as part of the sepsis bundles.
- Sepsis-3 definitions lactate levels were included in defining patients with septic shock.
- Serial lactate measurements are useful in monitoring treatment effectiveness to various therapeutic interventions, and therefore, is recommended in the sepsis bundle for septic shock, especially when the initial level is high.
- Monitoring lactate clearance through serial measurements has been demonstrated to be a useful predictor of morbidity and mortality. Patients with a decrease in an initially elevated lactate level within 24 hours have significantly better outcomes than patients whose lactate remains elevated.
- Elevated levels of lactate are not considered specific for either the diagnosis of sepsis, or predicting mortality, unless thoughtfully coupled with the overall clinical picture.
- Point-of-Care (POC) determinations: results in 15 seconds, easy to perform, small volume, good correlation with central lab.

Procalcitonin

- PCT is the only analyte approved by the FDA for the assessment of risk for developing severe sepsis in critically ill patients upon their first day of admission to intensive care units. Severe sepsis is no longer part of the sepsis 3 definition.
- Lactate and C-Reactive Protein are FDA-approved but not specifically for sepsis.²
- PCT is closely associated with inflammation, but not completely specific for infection. Evidence has shown that it may be elevated in a number of disorders in the absence of infection, especially following trauma.² Using a single concentration value for the diagnosis or prognosis of sepsis is not practical.
- PCT should always be interpreted carefully in the context of medical history and other clinical information as recommended in the Surviving Sepsis Campaign.²
- Immunoassays ~ 50 min to produce results.

Surviving sepsis in Veterinary Medicine: Chapter 3- Diagnosis and Treatment Challenges in Small Animals

Introduction.

Keypoints

- Clinical syndrome of local expanding to systemic inflammation in response to infection or injury.
- Alterations in the regulation of vasomotor tone, increased vascular permeability, dysfunctional microcirculation and coagulation abnormalities.
- Treatment should be directed towards resuscitation, early administration of liquids and antibiotics if bacterial sepsis is suspected, within 1 hour or less.
- Untreated sepsis leads septic shock:
 - Hypotension, vascular leak and microvascular dysfunction
 - Global oxygen debt, organ failure and death.
- What dogs and cats present?
 - Vague signs of illness.
 - Possibly conflicting information from owners.
- What to do?
- Immediately perform diagnostics and treatment bundle. Time matters.
- Decision?
 - Treat dogs or cats symptomatically as outpatients or,
 - Recommend hospitalization or referral.
- What do we have to keep in mind if we suspect sepsis?⁵⁰
- Measure lactate level.
- Blood sample for cultures.
- Administer broad-spectrum antibiotics.
- Begin rapid administration of crystalloids.
- Apply vasopressors if patient is hypotensive or after fluid resuscitation.

Diagnosis.

Initial steps

- Patient history.
- Physical examination.
- Laboratory work (Stat, BGA, blood cultures).
- Preliminary diagnostic findings = pt history + physical + lab.
- Watch for fluid overload.
- Options:
 - Suspect and confirm systemic inflammatory response.
 - Suspect and confirm sepsis, and septic shock.

Patient history & physical

- History:
 - Diarrhea, lethargy, loss of appetite, mental depression, vomiting (both cats and dogs).

- Physical exam:
 - Focus on vitals and BAR (bright and alert behavior)!!
 - Temperature.
 - Heart rate.
 - Respiratory rate.
 - Weight (fluid therapy).

Table 5. Risk factors for SIRS.⁵¹

Non-infectious Systemic Inflammatory Response (SIRS)	Infectious Systemic Inflammatory Response (SIRS)
Burns, chemical aspiration	Anaerobic bacteria, fungi
Heatstroke, Immune-mediated disease	Products of gram negative or gram positive bacteria
Ischemic organ necrosis (eg, splenic torsion)	Protozoa and viruses
Neoplasia, Pancreatitis, Trauma	

Symptom comparison: SIRS vs Septic Shock.

Table 6. SIRS vs Septic Shock.⁵²

SIRS	Septic Shock
Fever	Variable body temperature
Tachycardia	Tachycardia or bradycardia
Tachypnea	Tachypnea
Lethargy and dull mentation	Stuporous mentation
Injected mucous membranes	Pale or grey mucous membranes
< 1 sec capillary refill time	> 2 sec capillary refill time
Bounding pulses	Weak or absent pulses
Dehydration	

- Findings at physical examination⁵¹.

Table 7. Findings at physical examination: comparison Dogs vs Cats

Findings	Dogs	Cats
Early or hyper-dynamic phase	Yes	No
Bounding pulses	Yes	No
Brick red mucous membranes	Yes	Yes
Fever	Yes	No
Tachycardia	Yes	Yes
Tachypnea		
Late phase or advanced disease progression	Yes	Yes
Hypotension	Yes	Yes
Hypothermia	Yes	Yes
Pale mucous membranes	Yes	Yes
Weak pulses		

Laboratory work⁵³

- Venous blood gas analysis (BGA) and/or lactate.
 - Send out for bacterial and antibiotic sensitivity.
- Complete blood count (CBC).
- Biochemistry profile.
 - Stat if you are equipped.
- Urinalysis.
- Clotting profile.
 - PT, aPTT.
 - +/- fibrinogen.
 - +/- D-dimers, fibrin degradation products (FDPs.)
- Which samples can we use for lab work?
 - Blood.
 - Bronchoalveolar lavage fluid or endotracheal/transtracheal wash fluid
 - Joint fluid.
 - Peritoneal effusion (increased fluid entering the cavity or decreased removal).
 - Pleural effusion.
 - Urine.

Treatment.

The Bundle Concept

- Survival rates have improved through the use of treatment bundles.
- “Bundle of care is a group of therapies that, when instituted together, result in better outcomes than if each individual component were to be implemented alone.”⁵⁴
- Bundles have proven efficacious in reducing sepsis mortality.⁵⁵
- Brief bundle components.⁵⁶
 - Lactate-
 - Samples for cultures-

- Early antibiotic administration-
- Hypotension treatment with fluids and vasopressors-
- Target central venous pressure and ScvO₂ (oxygen debt)-

Overview

- Initial hemodynamic stabilization.
- Alleviating the underlying cause.
- Intensive care support.

Organization:

- Initial resuscitation (1-3 hours) and fluids.
- Antibiotic therapy, debridement and blood Cultures.
- Long term management (>3 hours)

Key points on treatment and resuscitation and of SIRS, sepsis and septic shock

- Time: 0 or immediate as patient checks in
 - Identify
 - Confirm suspicion
- Time: 20 min?
 - Resuscitate & Reassess
 - O₂, IV fluids, antibiotics
- Time: 30 min?
 - Investigate and confirm suspicion
- Time: 1 hr?
 - Disposition
 - Set limitations
 - Surgery
 - Pt evolution
 - Hospitalize, ICU

Resuscitation (See Table 8)

The seven bundle elements within 1 hour.⁵²

- Measure serum lactate
- Obtain blood cultures prior to antibiotic administration
- Broad-spectrum antibiotic within 1hr
- Treat hypotension and/or elevated lactate with fluids
- Administer vasopressors for hypotension not responding to initial fluid resuscitation to maintain mean arterial pressure (MAP) >65 mmHg. In the event of persistent hypertension despite fluid resuscitation (septic shock) and/or lactate >4 mmol/L, maintain adequate central venous pressure (CVP) and central venous oxygen saturation.
- Achieve a CVP of >8 mmHg and central venous oxygen saturation (ScvO₂) >70% or mixed venous oxygen saturation (SvO₂) >65%. Resuscitation.

Initial resuscitation: restoration of hemodynamic stability

- Aims of the goal-directed therapy.
 - Central venous pressure.
 - Mean arterial pressure.
- Place an IV catheter.
 - Cephalic or saphenous initially.
- Administer isotonic crystalloid boluses.
 - LRS, Plasmalyte-A, Plasmalyte-148, Normosol-R.
 - 20-25 mL/kg IV over 15 minutes.
- Re-assess perfusion parameters.
- Continue until perfusion restored up to 80-100 mL/ kg.
- Best to use balanced electrolyte solutions (NaCl reduces renal flow).

Table 8. Response to Fluid Resuscitation.

Parameter	Target
Heart rate	80-140 bpm
Respiratory rate	18-24/min
Pulses	Palpable femoral & dorsal pedal
Systolic BP	100-120 mmHg
Mean BP	70-80 mmHg
Lactate	< 2 mmol/L
Urine output	> 1 mL/kg/hour
Mentation	Responsive

Stabilization.⁵⁷

Patients with SIRS/sepsis may be resuscitated and supported with one or more of the following fluids:

- Crystalloids and blood component therapy: Isotonic, hypertonic or synthetic
- Isotonic crystalloids (very important in treatment).
 - Pts with severe cardiovascular issues: administer and repeat small 10 to 20 mL/kg boluses and monitor pt response to each bolus.
- Important: do not overload leading to pulmonary edema.
- Monitor improvement: pulse quality, decreased lactate level decreased heart rate, improved mentation.
- Synthetic colloids.
 - Useful in pts with SIRS/sepsis, especially if they are hypo-proteinemic.
 - Large molecules that not leave the vascular space, help pull fluid to, and keep it within, the vascular space.

- Hydroxyethyl starch solutions –
 - 20 mL/kg Q 24 H in dogs and cats.
 - dogs should be delivered in 5-mL/kg increments up to 20 mL/kg.
 - cats, 3- to 5-mL/kg increments up to 10 mL/kg.
 - Infusion rate should be constant to 1 to 2 mL/kg/H can be administered to increase oncotic. pressure in stable hypo-proteinemic patients.
- Blood products
 - Red blood cell (RBC) transfusion.
 - Recent blood loss (or RBC lysis).
 - Requirement for general anesthesia and surgery, packed cell volume (PCV) below 25% to 30%.
- Frozen products.
 - Evidence of bleeding, prolonged clotting times.
- Lyophilized Products (albumin).
 - Severely hypoalbuminemic with concurrent hypovolemia and hypotension.
 - Doses.
 - ✓ Packed RBCs: 10 to 15 mL/kg.
 - ✓ Fresh whole blood: 20 to 25 mL/kg.
 - ✓ Fresh frozen plasma: 15 mL/kg.
 - ✓ Lyophilized Albumin (canine): 800-884 mg/kg 6 hrs.

Antibiotics.

- Key points:
 - Early empiric therapy will improve survival rates.
 - Initiate broad spectrum while waiting blood culture results.
 - Selected antibiotics should be effective against gram-positive, gram-negative, and anaerobic bacteria.
- Typical first line:
 - Ampicillin/Unasyn + Amikacin.
 - Ampicillin/Unasyn + Enrofloxacin.
 - Cefazolin + Cefotaxime.
 - Cefoxitin.
 - Clindamycin + Enrofloxacin.
- How long to treat: 1-2 weeks

Table 9. Antibiotics, dosages and spectrum.

Drug	Dose	Spectrum
Amikacin	15-30 mg/kg IB q24 h	Gram Pos: + Gram Neg: ++
Ampicillin Unasyn	20-30 mg/kg q 8 h	Gram Pos: + Gram Neg: + Anaerobes: +
Cefazolin	20-30 mg/kg q 8 h	Gram Pos: + Gram Neg: ± Anaerobes: ±
Cefoxitin	20-30 mg/kg q 6-8 h	Gram Pos: + Gram Neg: + Anaerobes: +
Clindamycin	11-22 mg/kg q 12 h	Gram Pos: + Gram Neg: + Anaerobes: +
Enrofloxacin	10-20 mg/kg q 24 h	Gram Pos: ± Gram Neg: ++
Metronidazole	10-15 mg/kg IV q 12 h	Anaerobes ++

Monitoring the patient.

- Sepsis management bundle.⁵²
- Vital signs.
- Blood pressure.
- ECG.
- Pulse oximetry.
- Lab work.
 - PCV, TP, BGA, Lactate, Electrolytes q 6-12 h.
- Pain score.
- Nursing
 - Change positioning rotate recumbency.
 - Passive range of motion.
 - Head above bed 30o.
 - sternal, or semi-sternal positioning.
 - Nebulization & coupage if pneumoni.
 - Wound/incision management.

Prognosis.^{58,59}

- Overall mortality = 47%
- Dogs without MODS = 25%
- Dogs with MODS = 70%
- ScvO₂ and base deficit are useful in predicting the prognosis of dogs with septic shock
- Animals with a higher ScvO₂ and lower base deficit at admission to the ICU
- have a lower probability of death.

Conclusions.

- SIRS and Sepsis can be very serious conditions with a cautious to poor prognosis.
- Rapid diagnosis and treatment with fluid resuscitation and antibiotics are crucial.
- If required and ASAP, surgical intervention or other source to control underlying conditions.
- Post-operative care and monitoring are intensive and expensive.
- The development of septic shock and requirement for vasopressors are poor prognostic indicators.
- Early Goal-Oriented-Therapy (EGOT) according to bundles may improve survival rates.

What do we have to keep in mind if we suspect sepsis?

- Measure lactate level and blood sample for cultures.
- Administer broad-spectrum antibiotics, and begin rapid administration of crystalloids.
- Apply vasopressors if patient is hypotensive or after fluid resuscitation.

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