Literature review

Veterinary perspective of the renal system in sepsis

Beatriz Gasser*[1] ✉ iD M.Sc; Ricardo A. Ramirez-Uscategui[2] ✉ iD Ph.D; Marjury Maronezi[1] ✉ iD Ph.D; Ana Rodrigues-Simões [1] ✉ iD Ph.D; Luiz Pérez-Gomes[1] ✉ iD Esp; Letícia Pavan[1] ✉ iD M.Sc; Marcus Rossi-Feliciano[1,3] ✉ iD Ph.D.

[1]Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Faculdade de Ciências Agrárias e Veterinárias, Departamento de Patobiologia e Teriogenologia Veterinária, Jaboticabal, São Paulo, Brasil.
[2]Universidade Federal dos Vales do Jequitinhonha e Mucuri (UFVJM), Instituto de Ciências Agrárias, Unaí, Minas Gerais, Brasil.
[3]Universidade Federal de Santa Maria, Departamento de Clínica de Grandes Animais, Santa Maria, Rio Grande do Sul, Brasil.
*Correspondence: beatrizgasser@hotmail.com

ABSTRACT

This paper aims to review, discuss and define the factors associated with development of kidney injury in patients with sepsis, diagnosis alternatives, therapy and prevention, offering to the veterinarians an updated guide to improve the prognosis of sepsis patients. Sepsis is a clinical condition that leads to systemic complications and to multiple organ dysfunction syndrome (MODS), mainly due to poor tissue perfusion in humans and animals. Acute kidney injury (AKI) is considered the most frequent and lethal among organ complications secondary to sepsis, however the etiology of AKI in patients with sepsis is complex and multifactorial and not completely elucidated. Early diagnosis of AKI is difficult and consequently the treatment is not very successful, due to hemodynamic aspects. The ultrasonography seems to be a promising exam for early diagnosis of this lesion, especially with the advent of contrast-enhanced ultrasonography (CEUS) technique, which makes possible to detect microcirculation changes in the renal parenchyma, opening the door for a variety of physiological, clinical and therapeutic applications, however, studies proving CEUS accuracy for early detection of renal damage related to sepsis in humans and animals are still necessary.

Keywords: Acute kidney injury; infection; inflammation; multiple organ failure; perfusion; renal blood flow (*Source: DeCS*).

RESUMEN

Esta revisión tiene como objetivo discutir y definir los factores asociados con el desarrollo de lesión renal en pacientes con sepsis, alternativas de diagnóstico, terapia y prevención, ofreciendo a los veterinarios una guía actualizada para mejorar el pronóstico de los pacientes con sepsis. La sepsis es una condición clínica que conduce a complicaciones sistémicas y al síndrome de disfunción multiorgánica (MODS), principalmente debido a una pobre perfusión tisular en humanos y animales. La lesión renal aguda (LRA) se considera la más frecuente y letal entre las complicaciones orgánicas

secundarias a la sepsis, sin embargo, la etiología de la LRA en pacientes con sepsis es compleja, multifactorial y no está completamente elucidada. El diagnóstico temprano de LRA es difícil y, en consecuencia, el tratamiento no es muy exitoso debido a los aspectos hemodinámicos. La ecografía parece ser un examen prometedor para el diagnóstico precoz de esta lesión, especialmente con el advenimiento de la técnica de ecografía contrastada (CEUS), que permite detectar cambios en la microcirculación de el parénquima renal, abriendo la puerta a una variedad de componentes fisiopatológicos, aplicaciones clínicas y terapéuticas, sin embargo, los estudios que demuestran la precisión de CEUS para la detección temprana de daño renal relacionado con sepsis en humanos y animales aún son limitados.

Palabras clave: Flujo sanguíneo renal; infección; inflamación; insuficiencia multiorgánica; lesión renal aguda; perfusión (*Fuentes: DeCS*).

INTRODUCTION

Sepsis is currently defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection (1) and is a clinical condition that causes high morbidity and mortality in humans and animals. A study conducted by the American College of Veterinary Emergency & Critical Care (ACVECC) estimated the incidence of this syndrome around 6 to 10% in dogs and the survival rate between 25 and 50% (2). This condition causes systemic complications mainly due to poor tissue perfusion and may lead to the development of multi-organ dysfunction syndrome (MODS), which affects approximately 50% of dogs with sepsis and increases the mortality rate by up to 70% (3).

Acute kidney injury (AKI) is considered the most frequent and lethal organ complications secondary to sepsis, occurring in 30 to 50% of affected humans (4,5,6,7), 32,5% of cats, associated with increased risk of death (8) and in 12% of dogs, of which only 14% survive (3,9). However, the physiopathology of this lesion is not completely elucidated, being associated with changes in renal blood flow (RBF), renal microcirculation or even by factors involved in the inflammatory response (6,10).

Considering the high incidence of renal damage in patients with sepsis, the limited information available about its physiopathology, diagnosis, treatment and the high morbidity and mortality rates, the objective of this review is to carry out a bibliographic survey to discuss and define the factors associated with development of kidney injury in patients with sepsis, alternatives for diagnosis, therapy and prevention, offering to the veterinarians an update and a guide tool to improve the prognosis of sepsis patients.

PHYSIOPATHOLOGY

The etiology of AKI in patients with sepsis is complicated and multifactorial. The kidneys have a complex vascular structure that originates from the renal artery, enters the renal parenchyma and branches into a network of vessels along the cortex and medulla, reaching the glomeruli where blood filtration is performed (11). The normal renal blood flow is accentuated, since the kidneys receive approximately 25% of the abdominal aorta blood flow (11) and several diseases can affect the perfusion of these organs, either by alteration of the total renal blood flow (RBF) or intra-renal hemodynamics (12). Although there is strong evidence to infer that it is triggered by a drop in renal blood perfusion (4), experimental studies in animal models evaluating the RBF have resulted in controversial conclusions (13). Recent studies have described that sepsis may be associated with increased RBF and still lead to injury of these organs, allowing to infer that this phenomenon may be caused by changes in renal microcirculation (6,10).

These facts indicate that the isolated evaluation of the RBF cannot reliably explain the development of this lesion (13); probably due to the complex nature of renal vascularization (6) and suggests that AKI in patients with sepsis may be associated with changes in renal blood perfusion (4), intra-renal circulation, or even by the association of other important factors such as inflammatory response (6,10), endothelial dysfunction, infiltration of inflammatory cells, glomerular thrombosis or tubular obstruction by necrotic cells and cellular debris (13).

In bitches affected by piometra caused by *E. coli*, one of the main causes of sepsis in canines, it is suggested that renal damage is consequence of chronic stimulation of the immune system by this bacterial agent, inducing formation of

circulating immunocomplexes that precipitate in the glomeruli. This dysfunction can affect the kidneys (glomeruli or proximal tubules), being a transient condition in most cases (14).

Severe sepsis is characterized by reduced number of functional capillaries and increased blood flow heterogeneity. Ischemic component wasn't found in renal artery flow, however, was evidenced a deficiency in the microcirculation distribution of the renal cortex, with the presence of micro-ischemic areas (15). To exemplify these changes, a renal hemodynamics model in mice with experimental endotoxemia, has been demonstrated heterogeneous oxygen distribution in renal microcirculation, which cannot be detected using traditional techniques, that provide only systemic oxygenation estimative as PaO_2 and SaO_2, and this microcirculatory alteration lead to severe renal dysfunction (16).

These changes can be explained because during sepsis endothelial changes play a key role in microcirculation dysfunction: circulating inflammatory cytokines lead to endothelial activation, altered the pro and anti-coagulant balance, increased the expression of endothelial adhesion molecules and the formation of microvascular thrombi in several organs, including the kidneys. In addition, activated leukocytes increase their adhesion to the endothelium by up-regulation expression of integrin receptors and may contribute to impairment microvascular flow and increase pro-inflammatory cytokines release within the capillaries, creating a vicious cycle in the inflammatory response (17).

Endothelial dysfunction is characterized by impairment endothelium-dependent vasorelaxation and increased reactivity to vasoconstrictive agents, which nitric oxide (NO) is highlighted as one of the main contributors to this endothelial injury. Endothelial NO-derived of NO-synthase (eNOS) prevents vascular dysfunction by a direct vasodilatory effect, that inhibit platelet aggregation and leukocyte activation. Inhibitors of eNOS activity have been related with increased organ ischemia. Concomitantly, during sepsis occurs tetrahydrobiopterin depletion, a necessary substrate for eNOS synthesis, contributing to its reduction and consequently, exacerbating multi-organ ischemia. The ischemic-induced activation of inducible-NO-synthase (iNOS) by the leukocytes, vascular smooth muscle cells and epithelial tubular cells also participates actively to vascular dysfunction (17). Erythrocytes may

also play a role in regulating microcirculation blood flow, by their ability to release NO in the presence of hypoxia and thus induce vasodilatation (18). An experimental sepsis model in rats demonstrated that during the initial phase, renal damage consisted in diffuse structural alterations of renal corpuscles and glomerular epithelium components, leading to increased permeability to albumin (19).

It has been demonstrated in a rat model with lipopolysaccharide (LPS)-induced endotoxemia, that LPS lead to an increase in IL-6, IL-10, and TNF-α plasma levels and leukocyte infiltration in both peritubular and glomerular areas, beyond the expression of iNOS, however the early fluid resuscitation can prevent hypotension reducing renal inflammatory, but not systemic inflammatory activation, suggesting that endotoxemia-induced hypotension leads to an ischemia–reperfusion injury that potentially activate the inflammation response in the kidneys (15).

DIAGNOSIS

The diagnosis of AKI is based on a sudden drop in glomerular filtration rate (GFR), clinically detected by increase in serum creatinine concentration as a waste product of metabolism (20). However, serum creatinine is a late and unspecific marker of AKI, since this parameter is influenced by extra-renal factors such as changes in muscles, malnutrition associated with hospitalization or disease, liver function and gastrointestinal elimination (20,21,22,23). A study compared nephrectomized and sepsis-induced rats with nephrectomized non-septic rats, finding that septic animals had lower serum creatinine levels, indicating that sepsis reduces creatinine production and its use as a single marker may underestimate renal dysfunction in sepsis (20).

There are many urinary biomarkers development to identify early the renal dysfunction, among them: albumin, N-acetyl-β-d-glucosaminidase (NAG), gamma-glutamyl transpeptidase (GGT), alkaline phosphatase (24). However, the most widely investigated as serum and urinary biomarker in humans were: Neutrophil gelatinase-associated lipocalin (NGAL), IL-18 and cystatin C (25). NGAL is a protein expressed by neutrophils and tubular renal cells, upregulated by renal tubular ischemia and described as a

great sensitive and specific biomarker for AKI prediction in dogs (26). Another interesting biomarker associated with renal function during sepsis in humans is the symmetric dimethylarginine (SDMA), the elevation of this marker proved to be related to worse immune and vascular responses (27). In dogs, SDMA allowed to identify AKI and chronic kidney disease, but could not differentiate between them (28) and a preliminary study in critically ill dogs, found no difference in SDMA concentrations between critically ill and healthy animals (29).

There are physiopathological differences between septic and non-septic AKI that also influence other plasmatic and urinary biomarkers used for diagnosis, as occurs for example with urinary IL-18, which is mostly excreted in septic AKI patients and predicts kidney function deterioration approximately 24 to 48 hours before routine clinically signs of AKI (13).

Early diagnosis of AKI in septic patients is crucial, once the rapid introduction of an appropriate therapeutic improves survival rate and, additionally, because supportive treatments are often nephrotoxic and can aggravate the renal injury (e.g. antibiotics and vasopressors therapy with inadequate fluid resuscitation) (30). As discussed, AKI development is usually associated with RBF decrease leading to hypoperfusion, ischemia and consequently, to acute tubular necrosis (ATN) (31). However, several studies indicate that sepsis-related AKI may result from: the hypodynamic state discussed previously leading to renal hypoperfusion and which apparently is more common in less critical patients; or from pro-inflammatory hyperdynamic state in which inflammatory and pathogen derived molecules, such as endotoxins, lead to renal lesion generally associated to RBF increase and which apparently is more common in critically ill patients (32,33).

With these precepts, it would be expected that acute tubular necrosis (ATN) would be the most frequent renal lesion in patients with sepsis-related AKI and, although renal biopsy for histopathological analysis of this tissue is the gold diagnostic tool to identify this lesions, its invasiveness and high risks limit clinical application. Nevertheless, some experimental data in animals support the assertion that there is heterogeneity in histological lesions associated to sepsis-related AKI, ranging from imperceptible changes to severe tubular necrosis in most of studies carried out (31). To list some

of the findings described by these studies: the kidneys of sepsis dogs were evaluated, finding generalized vascular congestion, sometimes associated with renal tissue hemorrhage, without ATN evidence (34). Another study evaluated post-mortem kidneys of 67 people who died of sepsis and although they found focal tubular lesion in 78% of the kidneys, most tubular cells were normal (35). It has been also evaluated histologically kidneys of pigs with sepsis induced AKI, finding a slight vacuolization of the tubular cells, with no signs of necrosis (36).

In view of the importance of RBF in the physiopathology of sepsis-related AKI, is possible to consider that the assessment of this parameter could help in the early identification of AKI. However, experimental studies that evaluated renal artery blood flow in animals in which sepsis has caused AKI presented discrepant results such as reduction, no alteration or even elevation of this parameter (13). Due to these results and the complexity of renal microcirculation, researchers attempted to evaluate hemodynamics in the renal microcirculation, with very promising results, although the techniques used for these studies were extremely invasive, at risk and requiring anesthesia (15,17,37,36,38), resulting in poor clinical applicability.

Non-invasive evaluation of renal perfusion can be performed by image techniques. Total renal blood flow can be evaluated by spectral Doppler exam and allows assessment of the presence, direction, and velocity of blood flow, however, this method does not allow to verify renal hemodynamics at capillary level (12). In a study using color Doppler, reduction in renal perfusion and increase in resistivity index of renal arteries were observed in bitches with pyometra, when compared to results obtained in the same animals seven days after ovariohysterectomy (39). Another study evaluated the Renal Resistive Index (RRI) by Doppler ultrasound and validated a RRI>0.74 as predictor of AKI in septic humans, describing this index as a useful technique to determine renal diseases (40,41). In fact, an experimental model in dogs compared RRI, serum urea and creatinine, and urinary NGAL as early predictors of induced AKI indicated that RRI increase significantly from third day after induced AKI, whereas NGAL, urea and creatinine, showed a late increment and parallel between them (42).

The evaluation of renal microcirculation has already been described in humans and animals

as experimental models using portable video microscopy techniques, such as orthogonal spectral image, dark field image (SDF), CytoCam incident dark field images and laser Doppler technique of cortical region (13,17,36), however, the orthogonal spectral and lateral field image (SDF) present limitations such as the need of kidney exposure for evaluation and the access to renal capillary network region was describes as limited. It has been demonstrated in rats with sepsis through laser imaging and renal phosphorimetry, that fluid administration to prevent systemic hypotension and in attempt to increase the systemic oxygen distribution does not increase tissues oxygenation if the microperfusion and oxygen distribution are not suitable in these tissues (15). Another study using phosphorimetry did not detect alterations in the renal microcirculation partial pressure O_2, despite the presence of hypoxemic areas in the renal cortical microcirculation (16). It has been demonstrated an increase in venous partial pressure O_2 in endotoxemic dogs, associated with hypoperfusion and hypoxia of the renal cortex, suggesting that the presence of a convective oxygen shift may contribute to the development of hypoxia in these tissues (43). The renal microcirculation has been evaluated using contrast-enhanced ultrasonography (CEUS) and compared it with sublingual microcirculation parameters assessed by CytoCam incident dark field images, validating the use of CEUS to identify alterations in renal microcirculation resulting from septic shock induced in pigs, once CEUS variables accompanied the microcirculation parameters variations during septic shock and fluid resuscitation (38).

CEUS consists of a promising diagnostic technique that uses microbubbles as a contrast agent to increase echo refraction and quantify organic perfusion. This diagnostic capacity is due to its highly reflectivity, the microbubbles increases the Doppler signal, allowing the detection of streams difficult to detect by traditional methods. The microbubbles generally contain a high molecular weight gas (e.g. perfluorocarbon) and have a diameter of 1 to 7 μm, allowing their passage through the capillary beds, strictly in the intravascular space, without interstitial diffusion or excretion in urine and its use is safe, contraindicated in pregnant, newborns and patients with severe congestive heart failure, not associated to liver, heart or kidney toxicity and hemodynamic effects free (44). A study evaluated the safety of CEUS in 411 dogs and 77 cats, reporting minor transient

adverse events, such as vomiting and syncope in less than 1% of dogs and no adverse effect in cats, additionally, not correlated CEUS with risk of death (45). Having defined the applicability and safety of CEUS, it is important to report that for quality examination, the animal must be as quiet as possible for a few minutes, limiting the application to restless or excited animals, since pharmacological restraint alters the objective parameters.

Characteristics visualized by this method establish parameters related to tissues filling (44), in addition to measuring the peak of ultrasound enhancement (moment of greatest intensity), the time required to reach the peak (time to peak) and blood flow parameters. It has been demonstrated that CEUS can detect early and effectively renal microvascular changes in an ischemia reperfusion injury model in mice (46). This technique has been used in medicine for evaluated myocardial viability, detection breast neoplasms, intestinal ischemia, intestinal inflammatory disease, peripheral arterial disease, hepatic vascular diseases and to study the normal renal perfusion (44,47) and renal perfusion in transplant patients (48). In canines this technique has been described for the evaluation of normal perfusion in diverse organic systems and in humans it has been demonstrated the applicability of CEUS for the evaluation of renal infarction in a cardiopathic patient (49,50).

CEUS technique has been used to evaluate accurately the physiologic renal macro and microcirculation in dogs and opened the door for physiological and pathological studies using this method to estimate the renal perfusion in humans and animals (12). In this line of research, CEUS allowed to identify a 20% decrease in renal perfusion induced by angiotensin II infusion in healthy volunteers' persons (51). A study conducted in healthy dogs determined cortical and medullar time to wash-in, peak of enhancement and wash-out by CEUS, standardizing the renal perfusion parameters in this species (11). In a canine model of induced renal ischemia, aiming to determine the diagnostic usefulness of CEUS, it was identified alterations in renal perfusion parameters up to 30 days before detecting abnormalities in routine biomarkers used for AKI determination (52). These promising results regarding the diagnostic accuracy of the CEUS technique have made it suitable for evaluation of renal perfusion in critically ill human patients with sepsis and in the evaluation of transplanted kidneys (4,6).

An experimental study in pigs observed that sepsis-related AKI occurred without changes in renal blood flow or renal vascular resistance, but with significant reduction in cortical microcirculation (36). While other studies have reported that AKI in sepsis occurs in the presence of renal artery vasodilation and preserved renal blood flow (53). The efficacy of CEUS was evaluated to assess changes in renal perfusion of dogs with acute kidney injury comparing with healthy dogs and demonstrated that sick group showed increased medullary peak intensity and medullary area under the curve (54). Finally, a recent study in septic-AKI bitches derived from pyometra showed that the peak of contrast intensity in cortical region is able to identify hemodynamic changes as a trigger of AKI in 60% of the cases and presents a diagnostic accuracy above 80% for early identification of this lesion (55). Therefore, CEUS is considered a reliable technology that enables real-time bedside evaluation of the vasculature and microcirculation, which is essential for critical care. Despite all novel ultrasound techniques, serum and urinary biomarkers for early AKI detection, their accuracy in sepsis-induced AKI are not totally clear.

TREATMENT

The treatment of sepsis and its hemodynamic effects is based on fluid administration, seeking to promote an adequate intravascular volume, control blood pressure and consequently guarantee tissues perfusion and oxygenation, in an attempt to reduce the organic lesions resulting from these alterations (56). However, the fluid resuscitation in some cases seems ineffective to promoting renal oxygenation. One study compared the effects of immediate and late fluid administration on renal microcirculatory dysfunction of endotoxemic rats and noted an increase in renal perfusion, but not in its oxygenation, and the perfusion was more pronounced in animals receiving immediate fluid therapy, consequently, they concluded that immediate volume resuscitation does not prevent the activation of systemic inflammatory mediators, but reduces renal inflammation (evidenced by reduction in iNOS expression and glomerular leukocyte infiltrate), however, it does not prevent renal microvascular hypoxia (15).

The type of resuscitation fluid also seems to has an important role in sepsis AKI outcome and present data suggests that balanced crystalloid solutions may improve renal outcomes and

survivorship in septic critically ill patients (57). The use of vasoactive agents need further investigation, although noradrenaline remains the vasopressor of choice for preventing sepsis-induced AKI (58), since it has been shown during sepsis a significantly increased in global and medullary renal blood flow and restored renal vascular tone toward but not above normal (59).

More specific therapies to protect the renal system from sepsis-induced injury are not available at this time; and therefore it is indicated to follow the recommendations for early goal-guided treatment proposed by surviving sepsis campaign (56). However, it is important to note that in view of the limited availability of renal replacement therapy in veterinary medicine, the diuretics remain virtually the only alternative available to treat oliguric septic patients (60), further limiting our patients' survival expectations.

In view of the serious problems of sepsis-induced renal insufficiency in animals and humans, it can be inferred that sepsis-related AKI have a multifactorial character that seems to involve changes in renal microcirculation perfusion, as well inflammatory factors still poorly understood, leading to high death rates among septic critically ill patients. Furthermore, the applicability of serum and urinary biomarkers for the early detection of AKI during sepsis may not be very accurate, due to hemodynamic aspects. Therefore, the ultrasonography seems to be a promising and minimally invasive tool for early diagnosis of this lesion, especially due to the advent of CEUS technique, which makes possible to detect microcirculation changes in the renal parenchyma, opening the door for a variety of physiological, clinical and therapeutic applications, however, studies that proving CEUS accuracy for early detection of renal damage related to sepsis in humans and animals are still necessary.

Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

Authors contribution

All authors participated in the conception, review, writing, proofreading and approval this manuscript.

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