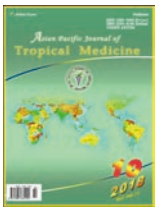

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Acute kidney injury in leptospirosis: Overview and perspectives

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ABSTRACT

Leptospirosis is a bacterial disease disseminated through the centuries in the whole world which causes symptoms that go from self-limited diseases to hemorrhagic manifestations and organ failure, including acute kidney injury (AKI), composing the severe disease known as the Weil's syndrome. Mortality rates varies according to the clinical presentation and usually increases when kidney injury is present, and is even higher in the setting of pulmonary hemorrhage. There are recent advances in the search for novel biomarkers of renal involvement and early detection of AKI in leptospirosis, as well as in its pathophysiology. We review in this article the clinical aspects of leptospirosis-associated AKI and the perspectives for future research.

1. Introduction

Leptospirosis remains the widest spread zoonosis worldwide, caused by an obligate aerobic spirochete of the *Leptospira* genus[1,2]. Renal involvement is a hallmark in severe forms, leading to acute kidney injury (AKI) and represents a major risk factor for death[3].

2. Historical aspects

Leptospirosis has been described by the first time more than one century ago, in 1886, by Adolph Weil in Heidelberg, Germany. He described 4 cases of men with jaundice, AKI and hemorrhagic phenomena, and this became known as the Weil's disease or Weil's syndrome[4]. In the ancient China, there were many reports of a disease affecting rice planters, whose clinical features were suggestive of leptospirosis[5]. In the XVII century, in New England and USA, there was an outbreak of hemorrhagic fever affecting Native Americans, manifesting with fever, headache, epistaxis, jaundice, and cutaneous lesions, as well as presented history of contact with rodents brought by ships coming from Europe[6].

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3. Ethical approval

All studies involving humans conducted by our research group were reviewed and approved by the Ethics Committee by our institutions and are in accordance with the Declaration of Helsinki and the Brazilian Guidelines for research with human being (resolution 466/2012).

4. Etiology

Leptospirosis is caused by bacteria from the *Leptospira* genus, which are obligate aerobic spirochetes with broad world distribution. The *Leptospira* species have been classified as *Leptospira interrogans* (*L. interrogans*; pathogenic) and *Leptospira biflexa* (*L. biflexa*; non-pathogenic saprophytes) for many years[5], but this classification seems to be currently inappropriate. Recently novel species of leptospiras have been described, classified in three main groups: saprophyte, pathogenic and intermediate, with dozens of species identified by now[7]. In 2010, some genomospecies acquired the status of species, resulting in 13 pathogenic bacteria with more than 260 serovariants and 6 saprophytic species with more than 60 serovars[1]. The term '*Leptospira*' comes from Greek, meaning thin (lepto) and coiled (spira), denoting the morphology of the bacteria[2]. The serovars of each species are determined by the agglutination of different antibodies. Each serovar is defined according to the organization of the antigenic epitopes of lipopolysaccharides (LPS) exposed in the bacterial surface[1]. The *Leptospira* species are determined according to the grade of genetic relationship, evaluated by DNA reassociation. The genetic mapping has been already described for many species[2].

5. Epidemiology

Leptospirosis has a wide geographic distribution, high incidence, and is the most important zoonosis in the world[8]. The transmission occurs by the exposure to water contaminated with urine of infected animals, thus the incidence peak is in the rainy seasons in tropical countries and in the period between summer and autumn in temperate weather regions[2]. Leptospirosis is classically associated with some risk factors, such as rural working, contact with contaminated water and mud, and water-sports practice. It is possible that migration phenomena that we are currently observing in different parts of the world due to political conflicts, wars, climate disasters and other reasons, may predispose infectious disease transmission and dissemination, and leptospirosis is on the list.

The annual incidence of leptospirosis is estimated on 1 million cases and 60 000 deaths per year[9]. The disease is endemic in tropical countries, especially in the Caribbean and Latin American countries, where the incidence is greater than 10 cases per 100 000 inhabitants per year[10]. In developed countries, the disease burden has decreased significantly. The urbanization reduced the contact with contaminated rural areas and the vaccination of domestic animals contributed to reduce disease transmission.

Brazil is among the 20 countries in the world with most cases of leptospirosis, with an estimated incidence of 12.8 cases per one million habitants[10]. According to data from SINAN/NET (Brazilian System for the Reporting of Notifiable Conditions), there were 20 810 confirmed leptospirosis cases reported in Brazil from 2010 to 2014, representing an average of 4 162 confirmed cases per year, and in the last 10 years a median of 3 600 cases each year is registered in the country[11]. The number of deaths due to leptospirosis in Brazil was 1 694 in the same period, representing an average of 339 deaths per year. Most cases were observed in the southeast region (7 457), followed by the south (6 030), north (3 929), northeast (3 141), and mid-west (253) regions.

6. Pathogenesis

The leptospiras infect the organisms by cutaneous lesions, wet skin, mucosal membranes, and conjunctivas or by the inhalation of microscopic particles (which is rare)[2]. After reaching the blood stream, the bacteria may affect all organs, especially the liver, kidneys, heart, and muscles, determining a vasculitis with endothelial lesions and inflammatory infiltrate. Infected human develop either a flu-like, usually self-limited febrile illness, or severe disease with renal, liver and heart failure, and eventually pulmonary hemorrhages that may be fatal, known as Weil's disease[12]. The severity of infection is related to age and immunocompetence of the human carrier[13].

Studies in animal models identified some virulence factors, however most of these factors do not have a well-defined role in the pathophysiology of leptospirosis. Significative advances were achieved by the identification of the interaction between the bacteria (*e.g.*, lig proteins) and host factors, including extracellular matrix proteins (*e.g.*, laminin, elastin, fibronectin, and collagen), proteins related to hemostasis (*e.g.*, fibrinogen and plasmin), and soluble mediators of complement resistance (*e.g.*, factor H and C4b-binding protein)[14]. Recent studies recently showed that recombinant Lig proteins can mediate *in vitro* interaction with fibronectin, fibrinogen, collagen, laminin, tropoelastin, and elastin[14].

The pathogenic mechanisms of leptospirosis may be divided into direct effects caused by *Leptospira* and the effects of the host immune response. An important virulence mechanism of the bacteria is its motility and its ability to move in viscous media, this being the main determinant in the initial phase of the infection[15].

Direct tissue lesion occurs due to the production of hemolytic toxins, which act as sphingomyelinases, phospholipases, and pore-forming proteins[2]. Leptospiras isolated from the kidneys of chronically infected animals demonstrate an increased amount of LPS, suggesting that the expression of LPS facilitates the permanence of the bacteria in renal tissues. The role of LPS and other (outer membrane proteins (OMPS) may be related to the activation of Toll-like receptors (particularly TLR2), which is responsible for the promotion of the humoral response, although TLR mechanism is to recognize pathogen pattern and activate innate system[13].

The main OMP expressed along the infection is LipL32, which

compromises directly proximal tubular cells and interstice by activation of pro-inflammatory genes and proteins, as iNOS, CCL_2/MCP-1, TNF- α and RANTES T cells[2].

Additionally, it was demonstrated that a glycoprotein (GLP), extracted from serovar *L. interrogans* Copenhageni, induced the production of cytokines, TNF-α and IL-10, by peripheral blood monocytes of healthy volunteers, besides its role in fibroblast toxicity of mice[16].

In the kidneys, the main finding is interstitial nephritis associated with intense leukocytic infiltrate of neutrophils and monocytes. Leptospiras may be observed within the renal tubules. Mild alterations are observed in the glomeruli, which suggest that this is the anatomic source for the proteinuria found in leptospirosis[5]. In chronically infected animals, the histopathologic findings in the kidneys and liver seem to be correlated with the presence of *Leptospira* in the affected tissues. In these same experimental studies, few leptospiras were found in the lung tissues, being the damage in these organs provoked mainly by the release of toxins from other tissues, such as the endothelium, which are carried to the lungs by the bloodstream[8].

7. Clinical manifestations

Leptospirosis is a disease of multiple clinical presentations. In general, the initial symptoms occur within 5 to 14 days of the infection[2,8]. The clinical course of the disease is classically biphasic. The initial phase, also known as acute or septicemic, lasts in average 1 week and is followed by the immune phase, characterized by the production of antibodies and the release of leptospiras in urine[8].

The clinical presentation of the initial phase is frequently non-specific, and the most frequent symptoms and signs are headache, anorexia, diarrhea, nauseas, vomiting, malaise, and myalgia, especially in the calves. Due to these clinical features, which represents 85%-90% of the infections[1], the disease is frequently sub-diagnosed or misdiagnosed as viral infections, such as dengue fever, influenza, and most recently, mainly in Brazil, Chikungunya and Zika virus infection.

In a study involving more than 200 patients with leptospirosis in Brazil, the main manifestations of the disease among patients admitted to the largest reference Infectious Diseases Hospitals in the region were: fever (96.5%), jaundice (94.5%), myalgia (92.5%), headache (74.6%), vomiting (71.6%), dehydration (63.5%) and chills (62.2%)[15].

The icteric phase usually lasts 1 week, and its resolution coincides with the appearance of antibodies. After this, approximately 10% of the patients will develop severe symptoms, with the possible occurrence of Weil's syndrome, meningitis and uveitis. IgM antibodies are commonly found during this phase and the severity of leptospirosis is associated with the intensity of the humoral immune response of the host[8,15].

Leptospirosis is an infectious vasculitis. In its severe form, patients may develop hemodynamic alterations secondary to hypovolemia, caused by the dehydration and direct effect of toxins that damage the vascular endothelium, increasing its permeability[17].

8. Renal involvement in leptospirosis

The kidneys are one of the most commonly affected organs by *Leptospira* infection. The renal involvement can vary from a subclinical course, characterized by mild proteinuria, microscopic hematuria and less extent leukocytes, with abnormal sediments in urine, to a truly acute kidney injury. AKI may occur in 20% to 85% of the patients during the second phase of the disease (septicemia)[3].

The two main mechanisms associated with the renal injury provoked by *Leptospira* are: (1) direct nephrotoxic action of the bacteria and the action of toxins inducing an immune response, and (2) indirect effects of the infection, such as dehydration, rhabdomyolysis and hypoxia due to hemodynamic alterations. The typical lesion is of tubulointerstitial nephritis, characterized by interstitial edema and dense local infiltrate with the predominance of mononuclear cells. The interstitial nephritis is the mainly renal alteration in leptospirosis, given that, even in those patients without AKI or tubular necrosis, is seen this pathological feature[3]. Therefore, interstitial nephritis is more common than acute tubular necrosis. The pathophysiology of leptospirosis-associated AKI is illustrated in Figure 1.

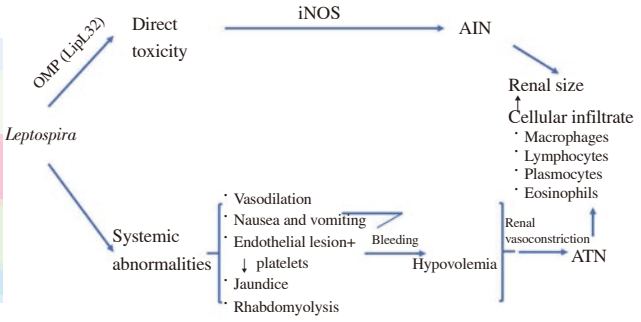

Figure 1. Pathophysiology of the acute kidney injury in leptospirosis. CCL2-Chemokine (C-C motif) ligand 2, iNOS– inducible nitric oxide synthase óxido nítrico sintetase indutível, OMP–outer membrane protein, MCP -1 Monocyte chemotactic protein-1.

The tubular characteristic is degenerative and affects mainly the proximal convoluted tubules. The intravascular volume depletion, causing vasomotor nephropathy may occur due to capillary lesions and subsequent loss of fluids and proteins. The outcome of this alteration is a rapid elevation in blood urea and creatinine, typical in AKI. In addition, jaundice and rhabdomyolysis are also associated with the development of AKI in leptospirosis[3].

The hemodynamic status and its alterations in the majority patients with severe leptospirosis are similar to those observed in patients with sepsis. Due to systemic vasodilation, aldosterone and antidiuretic hormone levels are elevated in the plasma. There is renal vasoconstriction and reduction of diuresis[18]. Tubular dysfunction, especially in the proximal convoluted tubules, are very common, even in the absence of AKI[19]. Three patterns of hemodynamic changes have been reported during severe leptospirosis. The first, which was the most common pattern, was decreased systemic vascular resistance (SVR) and mean arterial pressure, increased

cardiac index (CI), but normal pulmonary vascular resistance (PVR) and pulmonary capillary wedge pressure. The second pattern, which was found in patients with pulmonary complication, was normal SVR and CI, while increased PVR, and the last pattern, in the setting of hyperbilirubinemia, was normal or slightly increased SVR. There were decrease in CI and mean arterial pressure while there were no changes in PVR and pulmonary capillary wedge pressure[18].

Another early characteristic of the kidney injury in leptospirosis is the ultrasonographic findings, which shows enlarged kidneys with relatively preserved parenchymal echogenicity, indicating tubulointerstitial nephritis. The kidneys turn to their normal size after efficacious treatment of leptospirosis. The histopathological examination of renal tissue in leptospirosis demonstrates kidneys increased in size, with foci of tubulointerstitial nephritis and cellular infiltrates made of macrophages, lymphocytes, plasmocytes, and eosinophils[3].

The hallmark of the clinical presentation of AKI in leptospirosis is normo- and hypokalemia and non-oliguric patients. These are explained by the fact that the main *Leptospira*-induced injury occurs in the interstitium and tubules, where practically all K^+ filtered by the kidneys is reabsorbed, and glomeruli usually are spared by *Leptospira* sp.[12,13]. A study enrolling almost 200 patients evidenced non-oliguric patients in 67.4% of the cases and documented a 3 times increased mortality in the oliguric patients[3]. Kidney injury in patients with hyperbilirubinemia represents a severe form of the disease frequently accompanied by oliguria or anuria[2]. Recent report also showed that 75% leptospirosis patients had hypermagnesuria, whereas 50% of patients had decreased the threshold of tubular reabsorption of phosphate[20].

Recent studies research novel biomarkers for the early detection of renal dysfunction in patients with leptospirosis. The most consistent findings were high levels of angiopoietin-2, which show a significant association with complicated clinical course of the disease, marked by the occurrence of AKI, sepsis, and admission in ICU[21]. Additionally, endothelial lesion biomarkers, especially the glycocalyx-related ones, were associated with renal injury even in patients with mild AKI. A recent multicentric study evaluated uNGAL and pNGAL as promising biomarkers of AKI in leptospirosis[22].

Chronic leptospirosis may develop if *Leptospira* persists in the tubular lumen and interstitium as a continuum of AKI or even asymptomatic renal colonization with insidious clinical. Chronic *Leptospira* kidney infection may present with characteristic chronic tubu lointerstitial nephritis and interstitial fibrosis. *Leptospira* OMP's elicit tubular injury and inflammation through toll-like receptors-dependent pathway followed by in duction of chemokines and cytokines relevant to tubular inflammation. *Leptospira* OMP may also induce activation of the transforming growth factor-beta/Smad-associated fibrosis pathway leading to accumulation of extracellular matrix[23]. Ratet and contributors[24], by live imaging of bioluminescent *L. interrogans* in mice, revealed that use of antibiotics administered later post-infection were not efficient against *L. interrogans* and kidneys were the reservoir of leptospires at the chronic phase, noticed by mild and progressive fibrosis of the interstitium.

9. Jaundice

Jaundice is present in almost all cases of severe leptospirosis, being one of the key signals for the presumptive diagnosis of the disease. Biochemical studies in humans demonstrated that aspartate aminotransferase and alanine aminotransferase concentration in serum are moderately increased, being associated with a mild increase in the alkaline phosphatase levels in leptospirosis[25]. The exact mechanism is still uncertain. Most reports show alterations in the biliary excretion and intrahepatic cholestasis associated with jaundice[8]. A recent study performed in animals demonstrated spirochetal infiltration in the Disse space, migrating between the hepatocytes, intercellular junctions, and damaging biliary canaliculi. The destruction of these canaliculi coincides with the elevation in the levels of conjugated bilirubin, aspartate aminotransferase, and alkaline phosphatase in the blood, whereas the values of alanine aminotransferase and Gama-GT are only mildly elevated. In a study by our group, it was observed that the levels of direct bilirubin were associated with the occurrence of oliguria, demonstrating that the cholestatic alterations are correlated with the development of AKI[3].

10. Hemorrhagic manifestations

Hemorrhage is typically described in the lungs of patients with severe leptospirosis. The hemorrhagic lung syndrome is one of the classical manifestations of severe leptospirosis. The pathogenesis is still poorly understood, albeit the cause of the pulmonary hemorrhage is associated with the deposition of immune complexes and complement proteins in the alveolar septa[26]. Furthermore, a diffuse process of endothelial inflammation, similar to observed in sepsis, associated with autoimmune phenomena and platelet activation contribute to this clinical scenario. A study with 147 patients in the Indic Ocean region demonstrated an increase in the incidence of pulmonary involvement in leptospirosis in this region. The mortality was 12.9% and mechanical ventilation (*OR*=20.94) was an independent risk factor for death[26].

11. Diagnosis

The diagnosis is based on the clinical features and in the epidemiologic data, being further confirmed by laboratory tests. Due to the initial unspecific presentation, high clinical suspicion is necessary, since leptospirosis is frequently misdiagnosed as dengue, hantavirus hemorrhagic fever, viral or bacterial meningitis, malaria or viral hepatitis. One of the clinical tools which may help the physicians in the rural area to early diagnosis leptospirosis is a scoring system. For example, the THAI LEPTO score which based on the clinical parameters and simple laboratory tests. The simplified score was the summation of the odds ratio values as follows: hypotension=3, jaundice=2, muscle pain=2, AKI=1.5, low hemoglobin=3, hypokalemia with hyponatremia=3, and neutrophilia=1. The score showed the highest discriminatory power with area under the curve 0.82 (95% *CI*=0.67±0.97) on fever day

three and four[27].

The absence of adequate and practical laboratory tests is a key challenge for the diagnosis of leptospirosis. This issue contributes to the sub-diagnosis of the disease and is an obstacle to the comprehension of the natural history of the infection[28]. Culture and microscopic agglutination test (MAT) are the golden-standard tests for the diagnosis of leptospirosis, however they are not useful for the early diagnosis. *Leptospira* cultures are difficult, as the bacteria have insidious growth, so it is not used in clinical practice. The anti-*Leptospira* antibodies measured by MAT only begin to be detected after the second week of infection[5].

The currently available diagnostic tests are listed as following:

11.1. Direct observation

The leptospiras may be isolated in the blood, cerebrospinal fluid, and peritoneal dialysate fluid during the first 10 days of the disease. Urine may be sent for culture in the first week of the infection[2]. In the blood, the bacterial amount varies from 102 to 106 leptospiras per milliliter in the acute phase. The limit for the detection is determined as approximately 104 leptospiras per milliliter of blood or urine. Despite of being relatively cheap, this diagnostic modality requires a dark field microscope, which is seldomly available.

11.2. Nucleic acid–based rapid tests

It has been developed and tested in clinical studies, however it is still rarely used in clinical practice[2]. Few tests were validated for use in canines and humans[28]. PCR technique is precocious and sensitive, but its elevated cost and the necessity of high quality controls are the main difficulties to its application. The limit for detection in essays with PCR is approximately 100-1 000 bacteria per milliliter in blood or urine[28]. The positivity of PCR usually indicates that one of the pathogenic *Leptospira* species is present in the sample, but it cannot predict the serovar.

11.3. Isothermal methods

The loop-mediated isothermal amplification method has been demonstrated as the most promising isothermal method. However, costs are similar of real time PCR and it is not clear when these tests are going to be really economically competitive [28].

11.4. Rapid tests using antibodies

The surface recombinant proteins and lipoproteins of *Leptospira* have been used as antigens. In general, the antigens used in ELISA may not be recognized by the diversity of circulating strains and the sensitivity of the test is poor[5]. Despite the variety of performance found in ELISA, the studies show that this method detects the anti-*Leptospira* antibodies earlier than the MAT[28].

11.5. Other methods of rapid antibody detection

These methods include macroagglutination, immunofluorescence-based assays, indirect hemagglutination, latex agglutination, lateral flow assays and IgM dipstick. The conclusive laboratory method most commonly used for the diagnosis of leptospirosis is the MAT, however it has two main complicating factors: availability restricted to reference centers and the preferential collection of two blood samples with 2 weeks of interval in between them, with the objective of increasing its sensitivity. The results are considered as positive when the titles of antibodies are four times greater the reference range. In Sri Lanka, a study demonstrated sensitivity of 92% and specificity of 73% for MAT, considering positive results when the titles were 1: 800.

12. Treatment

The diagnosis and implementation of early adequate therapy are crucial aspects in the management of patients with leptospirosis. Supportive measures are essential and specific measures should be instituted, when indicated, as soon as possible. Several antimicrobial agents are active *in vitro* against leptospiras, such as penicillins, tetracyclins, streptomycin, azithromycin, cephalosporins, and others. A recent study evaluating 206 patients with leptospirosis admitted in hospitals demonstrated ceftriaxone as a protective factor for admission in ICU, suggesting that its use may prevent the severe forms of the disease[29]. However, the Cochrane group, in a meta-analysis performed in 2012, analyzing 7 randomized studies did not conclud to have enough evidence to recommend or disapprove the use of antibiotics in leptospirosis. In the patients, who survived until hospital discharge, antibiotic therapy reduced the extent of hospitalization in 2 to 4 days, albeit this result was not statistically significant. The choose of cephalosporin, doxycycline, or penicillin did not shown impact in mortality and the length of fever[30].

13. Genomics and perspectives

In the last 25 years, many strategies have been developed to increase the efficacy of the vaccines against leptospirosis. The genetic sequencing of the *L. interrogans* serotype Copenhageni performed in Brazil was one of the main determinants in this evolution[31]. Regarding the advances in the early diagnosis of the renal damage, the markers uNGAL and pNGAL were demonstrated as promising in the evaluation of the AKI in leptospirosis in a recent multicentric study[22], and in mild forms of leptospirosis AKI, biomarkers of endothelial lesion were associated with kidney damage[32].

Conflict of interest statement

The authors declare there is no conflicts of interest regarding this publication.

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