Leptospiral Nephropathy

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Summary: Leptospirosis is recognized as a globally re-emerging zoonosis. Interstitial nephritis is the principal feature of the disease. Leptospirosis-induced acute kidney injury typically is nonoliguric and includes hypokalemia. Tubular function alterations precede a decrease in the glomerular filtration rate, which could explain the high frequency of hypokalemia. Studies in human beings and animals have shown increased urinary fractional excretion of potassium and sodium, as well as an increased potassium/sodium ratio, suggesting increased distal potassium secretion caused by increased distal sodium delivery consequent to functional impairment of proximal sodium reabsorption. Confirming these findings, Western blot studies have shown lower renal expression of the sodium/hydrogen exchanger isoform 3 and of aquaporin 2, together with higher renal expression of the Na-K-2Cl cotransporter NKCC2, in infected animals. The severe form (Weil's disease) manifests as diffuse alveolar hemorrhage, pulmonary edema, acute respiratory distress syndrome, or a combination of these features, accompanied by acute kidney injury and can be highly lethal. Antibiotic treatment is efficient in the early and late/severe phases. For critically ill leptospirosis patients, the following are recommended: daily hemodialysis, low daily net fluid intake (because of the risk for pulmonary hemorrhage), and lung-protective strategies (low tidal volumes and high positive end-expiratory pressures after recruitment maneuvers).

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Leptospirosis is a zoonosis of global distribution, present on all continents.[1-3] It is caused by microorganisms of the genus *Leptospira*, which affect human beings and a wide variety of animals. Leptospirosis is recognized as a globally re-emerging infectious disease because of a marked increase in the number of cases, as well as in the frequency of outbreaks in Latin America and southeast Asia.[1-5] Although leptospirosis traditionally has been a disease of rural areas, major outbreaks have occurred in low-income communities within large cities.[1,3,6]

Leptospirosis has epidemic potential in tropical and temperate climates.[3] In Brazil, leptospirosis is endemic, and outbreaks occur during the rainy season, coinciding with localized flooding.[6] The real impact of the disease might be underestimated because many patients with leptospirosis are misdiagnosed as suffering from other infectious diseases, such as dengue or influenza. A few years ago, the International Leptospirosis Society created a surveillance network to register cases of leptospirosis at the global level (http://www.leptonet.net).

Leptospirosis can provoke a broad range of manifestations, from benign infection (characterized by nonspecific symptoms) to Weil's disease, which is a severe form of the disease that causes jaundice, hemorrhagic events, and acute kidney injury (AKI).[7-9] The disease is a common cause of fever in developing countries and continues to be a lethal infection. The mortality rate among patients with Weil's disease is more than 10%.[1]

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ETIOLOGY

The etiologic agent of leptospirosis is an obligate aerobic spirochete (leptospire) that is helical, flexible, and motile, measuring 6 to 20 μm in length and 0.1 μm in diameter.[1,2] Leptospires have a distinctive double-membrane architecture concomitantly presenting features of gram-positive and gram-negative bacteria.[2] The species *Leptospira interrogans* is divided into 2 complexes: *L interrogans*, which is pathogenic; and *Leptospira biflexa*, which is nonpathogenic and saprophytic. The *L interrogans* complex comprises 23 serogroups and approximately 210 serovars.[2,6,7]

TRANSMISSION

Transmission to human beings occurs through direct contact with blood, tissues, organs, or urine of infected animals, or through indirect contact when injured mucosa or skin comes into contact with contaminated water. The risk of transmission increases during the rainy season, when such water accumulates during flooding. A significant characteristic of leptospires is their ability to rapidly penetrate and disseminate to the various compartments of the host organism. In the renal tubules, they establish robust colonies.[1-6]

DIAGNOSTIC METHODS

The diagnosis of leptospirosis is based on clinical and epidemiologic data but is confirmed through laboratory tests. The most widely used method for diagnosing leptospirosis is the microscopic agglutination test (MAT), which is performed using 2 blood samples collected 2 weeks apart. The MAT results are considered positive when the antibody titers are 4 times higher than the reference value. The efficacy of the MAT was investigated recently by the International Leptospirosis Society, and the rate of false-negative results was found to be 13%.[1] Isolation of *Leptospira* requires specific media (Ellinghausen-McCullough-Johnson-Harris or Fletcher medium) and can take more than 4 weeks. Commercial anti-whole *Leptospira* IgM detection kits in enzyme-linked immunosorbent assay or rapid formats are used to provide presumptive confirmation of leptospirosis. However, their sensitivity is low (39%-72%) during the acute phase. Although diagnostic methods based on polymerase chain reaction have been developed, their use has been limited to referral laboratories.[10]

CLINICAL MANIFESTATIONS

The clinical manifestations of leptospirosis range from mild symptoms (anicteric form) to severe disease (icteric form, or Weil's disease).[2,3] The anicteric form is a self-limited disease and is the more common, accounting for 85% to 90% of cases. It is classically characterized by 2 phases. The initial phase lasts for 3 to 7 days. Infected individuals suddenly present with high fever, chills, and intense headaches, subsequently developing anorexia, diarrhea, nausea, vomiting, and malaise, as well as generalized myalgia, which is most pronounced in the calves. The fever typically is 38°C to 39°C and remits within 4 to 7 days after the initial appearance of the symptoms. In this phase, it is possible to isolate leptospires from blood samples. In some cases, the individual then experiences 1 to 3 fever- and symptom-free days, after which the so-called *immune phase* begins, and the symptoms return. This phase lasts from 4 to 30 days, and more severe symptoms, such as meningitis and uveitis, can occur. IgM antibodies are commonly found in this phase. Only approximately 20% of patients present the immune phase.[2]

In the city of Fortaleza, Brazil, 201 patients hospitalized with leptospirosis were evaluated between May of 1985 and December of 2006. The principal clinical characteristics were fever, jaundice, and myalgia (Table 1). The respective laboratory findings are presented in Table 2.

Cardiac involvement in leptospirosis is characterized by arrhythmias (atrial fibrillation being the most common), atrioventricular blockage, and myocarditis, although pericardium rub and effusion also can occur.[11] In a study involving 20 patients who were dying from leptospirosis, De Brito et al[11] observed interstitial edema, myocardial infiltration, acute coronary arteritis, and aortitis. The investigators also detected leptospiral antigens in the aorta and coronary arteries.

Table 1. Clinical Findings Presented by Patients With Leptospirosis in Fortaleza, Brazil

	n = 201
Age, y	38.9 ± 15.7
Sex	
Male, n (%)	159 (79.1%)
Female, n (%)	42 (20.9%)
Onset of symptoms to admission, d	7.1 ± 3.2
Length of hospital stay, d	11.0 ± 7.1
History of contact with rats	29.8
Mean blood pressure at admission	
SBP, mm Hg	108.9 ± 20.7
DBP, mm Hg	67.1 ± 14.8
Pulse, bpm	101.6 ± 17
Signs and symptoms (%)	
Fever	96.5
Jaundice	94.5
Myalgia	92.5
Headache	74.6
Vomiting	71.6
Dehydration	63.1
Chills	62.2
Calf pain	51.7
Diarrhea	42.3
Hepatomegaly	37.8
Anorexia	37.3
Oliguria	31.8
Tachypnea	32.3
Dyspnea	28.3
Crackles or rhonchi	22.9
Petechiae	20.4
Arthralgias	19.9
Hemoptysis	13.4
Hematemesis	12.9
Conjunctival suffusion	11.9
Edema	11.4
Disorientation	9.4
Flapping	5.4
Constipation	4.9
Splenomegaly	2.9
Seizure	1.0
Death, %	15.4

Mean ± SD or % shown.
Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure.

Weil's disease provokes potentially fatal hemorrhagic manifestations. Patients can develop significant hemodynamic abnormalities secondary to the hypovolemia caused by dehydration and the direct effects of leptospiral toxins that damage the vascular endothelium and increase permeability.[12] Hemorrhagic manifestations include ocular suffusion, petechiae, pulmonary hemorrhage, gastrointestinal hemorrhage, and hematuria. Thrombocytopenia is seen in more than 70% of cases. Hemorrhage has become recognized as the most serious manifestation of human leptospirosis, and reports of such hemorrhage are increasing worldwide.[13] Recently, Daher et al[14] showed that leptospirosis severity is associated with the intensity of the humoral immune response.

The principal finding involving the central nervous system is headache of sudden onset (in the initial phase). Meningitis is a common complication in the immune phase.

RENAL INVOLVEMENT

The reported incidence of AKI in severe leptospirosis varies from 40% to 60%. The kidney is one of the principal target organs of *Leptospira*. Leukocytes, as well as to a lesser extent erythrocytes, are seen in the urinary sediment of leptospirosis patients. Urinary protein excretion, when present, typically is less than 1 g/d. Bile pigments and granular casts also can be seen. Under dark-field illumination, leptospires can be seen in urine between weeks 1 and 4 of infection.[15]

Pathologic findings include foci in tubules and interstitium, the glomeruli usually being spared and presenting normal capillary loops. However, it is not uncommon to find protein within Bowman's space. Acute inflammatory cells are seen occasionally in and around the renal tubules. There also is mild to marked histiocytic infiltration. However, glomeruli maintain a normal aspect.[16] The basic pathologic alteration caused by the disease is interstitial nephritis. Interstitial edema and infiltration are observed, even in patients without renal failure or tubular necrosis. The infiltration can be diffuse or can be focused around the glomeruli and venules. The infiltrate consists primarily of mononuclear cells.[17] Silver and other immunohistochemical staining reveals large numbers of intact leptospires. Intact leptospires have been observed throughout the tubular basement membrane, among tubular cells, within the tubular lumens,

Table 2. Laboratory Findings During Hospital Stay in Patients With Leptospirosis in Fortaleza, Brazil

Laboratory Findings	Patients With Data	Mean ± SD or %
Hematocrit, %	183	32.5 ± 6.0
Hemoglobin, g/dL	181	10.6 ± 1.9
White blood count, $\times 10^3/mm^3$	159	14.37 ± 9.01
Platelet count, $\times 10^3/mm^3$	156	77.25 ± 67.92
Serum sodium level, mEq/L	196	135.4 ± 11.7
Serum potassium level, mEq/L	189	3.8 ± 1.2
Serum urea level, mg/dL	159	172.0 ± 90.6
Serum creatinine level, mg/dL	192	5.0 ± 2.9
Serum calcium level, mmol/L	60	8.0 ± 1.9
Serum phosphorus level, mmol/L	49	3.8 ± 1.0
Serum chloride level, mmol/L	32	103.1 ± 11.7
Prothrombin time, %	58	59.3 ± 30.0
AST level, IU/L	164	112.3 ± 106.4
ALT level, IU/L	165	88.5 ± 110.7
Direct bilirubin level, mg/dL	160	13.5 ± 9.8
Indirect bilirubin level, mg/dL	162	5.6 ± 5.2
Lactate dehydrogenase level, IU/L	67	693.7 ± 418.1
Creatine phosphokinase level, IU/L	56	702.1 ± 2638.0
Blood pH	102	7.37 ± 0.06
Blood HCO_3, mEq/L	89	19.49 ± 4.88
Urinalysis		
pH	110	5.7 ± 0.7
Hematuria	175	42.9%
MAT	106	75.5%

Abbreviations: AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCO_3, bicarbonate; MAT, microscopic agglutination test.

within the interstitium, and, in some cases and in limited numbers, within glomeruli. Fragments of spirochetes have been found within histiocytes, in the interstitium, and in tubules. Degenerated spirochetes are rarely found in the glomeruli.[12]

Leptospirosis-induced AKI typically is nonoliguric and often includes hypokalemia. Seguro et al[18] studied 56 patients with leptospirosis-induced AKI and found a higher frequency of nonoliguric renal failure. The investigators also found that morbidity and mortality were lower in those with nonoliguric AKI than in those with oliguric AKI. In addition, 45% of the patients were hypokalemic, and none were hyperkalemic. A prospective study involving 11 of those patients showed that the initially increased urinary fractional excretions of potassium and sodium (FEK and FENa) and the urinary potassium/sodium ratio, suggesting that distal potassium secretion increased owing to greater distal sodium delivery consequent to functional impairment of proximal reabsorption of sodium. By postadmission day 8, FEK and FENa had decreased.

Magaldi et al[19] inoculated guinea pigs with *Leptospira icterohaemorrhagiae* to evaluate renal tubular function, using clearance and microperfusion of isolated nephron segments. Although all animals with leptospirosis presented jaundice, inulin clearances were normal. Animals with leptospirosis presented higher FEK than did normal animals. High doses of furosemide were used to block sodium chloride reabsorption in the thick ascending limb of Henle's loop of the leptospirosis-infected animals, which subsequently presented FENa and FEK that were higher than those seen in normal animals treated with the same diuretic dose. In

the infected, furosemide-treated animals, the mean FEK increased from 26% to 136%, confirming that the distal tubular segments were intact, and that distal potassium secretion had increased.

The microperfusion studies performed by Magaldi et al showed that the medullary thick ascending limb of normal animals presented transepithelial potential difference and relative sodium-chloride permeability identical to those seen in that of leptospirosis-infected animals, indicating that this nephron segment was functioning normally. In the inner medullary collecting duct of animals with leptospirosis, osmotic water permeability, diffusional water permeability, and urea permeability did not increase in the presence of vasopressin, indicating vasopressin resistance in the inner medullary collecting duct, which might explain the high frequency of nonoliguric AKI in leptospirosis.[19] The relevant finding of that study was that tubular function alterations in leptospirosis precedes the decrease in glomerular filtration rate, which could explain the high frequency of hypokalemia in leptospirosis-induced AKI even in oliguric patients.

In a prospective study of 42 patients with acute lung injury in leptospirosis, most of them with AKI, a serum potassium level greater than 4 mmol/L was associated independently with mortality. Lower potassium levels were observed in survivors, suggesting that there is less renal dysfunction in this group. The higher potassium levels observed in nonsurvivors might have been provoked by more severe renal dysfunction or rhabdomyolysis. The association between creatinine phosphokinase levels and maximum serum creatinine levels in these patients suggests that rhabdomyolysis contributes to AKI and higher potassium levels in nonsurvivors.[20]

In hamsters infected with leptospirosis, Andrade et al[21] showed a significant decrease in the protein expression of the sodium/hydrogen exchanger isoform 3 (NHE3), which is expressed in the apical membrane of the proximal tubule. The decrease in protein expression of this sodium transporter can partially explain the polyuria and might completely explain the high FENa. Apical NHE3 plays an important role in reabsorption of sodium and fluid by the proximal tubule. As a consequence of the decreased NHE3 protein expression, fluid delivery to the distal nephron might be increased. The investigators showed a marked increase in Na-K-2Cl cotransporter (NKCC2) band density. This might represent a compensatory response to the greater sodium chloride and water delivery. The protein expression of the Na-Cl cotransporter and the α subunit of the epithelial sodium channel (α-ENaC), both regulated by aldosterone, were unchanged in the renal cortices of the infected animals. The investigators speculated that the renin-angiotensin-aldosterone system is not involved in this animal model of leptospirosis, although they acknowledged that these transporters might be unresponsive to aldosterone. Notably, the leptospirosis-infected animals evaluated in that study showed characteristics consistent with urinary concentrating defect (significant increases in urinary volume and significant decreases in urinary osmolality). The investigators found that leptospirosis down-regulated the expression of aquaporin 2 by 50%.[21]

Other tubular dysfunctions have been reported. Khositseth et al[22] found that 15 (75%) of the 20 leptospirosis patients studied presented hypermagnesuria, whereas 10 patients (50%) presented decreased tubular reabsorption of phosphate. Renal magnesium and phosphate wasting caused hypomagnesemia and hypophosphatemia in 9 and 3 patients with AKI, respectively. These abnormal findings significantly improved within 2 weeks after admission. The investigators also identified N-acetylglutamate and β2-microglobulin, which indicate proximal tubular dysfunction, in all 20 patients.[22]

Increased knowledge of leptospirosis-induced electrolyte disorders and polyuria is of immediate clinical significance because early diagnosis and correction of these electrolyte disorders can improve clinical outcomes for these critically ill patients.

Although rat kidneys are a major natural reservoir of infection, rats are relatively resistant to leptospirosis. Despite the importance of the rat in disease transmission, few reports have focused on the underlying pathology in this species. A broad range of morphologic alterations have been detected in the kidneys of captured

rats. However, interstitial nephritis was the only feature reproduced under experimental conditions. Faria et al[23] reported the renal pathology associated with spontaneous leptospiral infection in captured urban rats in Salvador, Brazil, as well as in experimentally infected Wistar rats. This comparison enables evaluation of the renal histopathology of infected rats under controlled conditions as well as in the urban environment where a diverse array of chemical and biological factors can influence the natural history of infection. Four months after the experimental infection, interstitial nephritis was seen in only 14.3% of the rats.[23]

The role of innate immune responses in protection against and pathogenesis of severe leptospirosis remains unclear. Toll-like receptors (TLRs) are now recognized as the major receptors for microbial pathogens on cells of the innate immune system. Recently, TLRs also were identified in many organs, including the kidney.[24] It has been shown that the TLR4 distribution along the nephron is affected by sepsis, and that this important receptor is accessible to systemic endotoxin. There are data indicating that TLR4 and these related proteins play roles in modulating the renal response to sepsis.[24] Viriyakosol et al[25] showed that intact TLR4 signaling contributes to the control of the tissue burden of *Leptospira* in nonlethal leptospiral infection. Natural mammalian reservoir hosts of leptospires generally do not develop severe pathology in leptospiral infection. It has been well documented that leptospires can persist for prolonged periods of time in the renal tubules of a wide variety of mammals. Therefore, the fact that the investigators found significantly higher numbers of leptospires in TLR4-deficient mice, particularly in the target organs mediating leptospiral disease (liver, lung, and kidney) and transmission (kidney), is novel and important.[25] In another study, the 32-kd lipoprotein from the outer membrane protein extract of the pathogenic *Leptospira santarosai* serovar *shermani* was introduced into mouse proximal tubule cells in culture, resulting in a dose-dependent stimulatory increase in monocyte chemoattractant protein-1, the cytokine regulated on activation, normal T-cell expressed and secreted, nitrite and tumor necrosis factor-α.[26]

RENAL FUNCTION RECOVERY AFTER LEPTOSPIROSIS-INDUCED AKI

Daher et al[27] evaluated the pattern of renal function recovery in leptospirosis. When and how renal function recovery occurs in leptospirosis-induced AKI was evaluated prospectively in 35 patients with leptospirosis-induced AKI during hospitalization and at discharge, as well as at 3 and 6 months after discharge. The investigators showed that, after the acute phase, the glomerular filtration rate returned to normal by postdischarge month 6, whereas proteinuria did not, reaching nephrotic levels, and was observed only during the acute phase. The decrease in urinary pH was reversed by postdischarge month 3. The recovery of these parameters was not influenced by the severity of AKI. Urinary concentration had not recovered by month 6, and the severity of AKI probably influenced the recovery of this parameter. The investigators concluded that, in leptospirosis-induced AKI, recovery of renal function, except for urinary concentration, is rapid and complete. Thus, although we had previously shown that the clinical pattern of leptospirosis seems to be turning into a more severe one, the renal functional recovery remains an early and almost complete one.[27]

AKI IN CHILDREN WITH LEPTOSPIROSIS

Leptospirosis is diagnosed less frequently in children than might be expected based on the level of exposure to hazards. This might be attributable to a failure to consider the diagnosis or to differences in the manifestations of leptospirosis in children. Marotto et al[28] studied 43 leptospirosis-infected children from 4 to 14 years of age. The investigators observed AKI in 79%, and, as in adults, the AKI was primarily nonoliguric. Eleven of the children had hypokalemia at admission. Only 2 children required dialysis during hospitalization. When compared with adult populations, children with leptospirosis-induced AKI had better outcomes. There was only 1 death among the children studied.[28]

More recently, an interesting case of anicteric leptospirosis-induced AKI and meningitis was described in a 19-month-old child whose family lived in an area that had been flooded 1 week before the onset of symptoms. Reversal of the AKI was obtained after antibiotic treatment and intravenous fluid therapy. This case report should alert pediatricians to the potential of leptospirosis in children with AKI and meningitis, particularly in endemic areas.[29]

PULMONARY MANIFESTATIONS IN LEPTOSPIROSIS

Worldwide, reports of pulmonary manifestations in leptospirosis have been increasing in recent years.[2,6] Leptospirosis-associated hemorrhagic pneumonitis has been highlighted in studies of the 1995 Nicaragua outbreak and of outbreaks at other locations.[30] Pulmonary involvement in leptospirosis ranges from 20% to 70%.[1,2] In 2006, in the Metropolitan area of São Paulo, the frequency of Weil's disease with pulmonary hemorrhage was 69%.[31]

Leptospirosis-associated hemorrhagic pneumonitis can manifest as cough, dyspnea, and hemoptysis, accompanied by radiologic abnormalities that range from focal interstitial infiltrate to diffuse alveolar infiltrate. More severe respiratory symptoms, such as respiratory failure caused by pulmonary hemorrhage, can be seen, resulting in high mortality rates.[32]

Leptospirosis is now recognized as a major cause of severe pulmonary hemorrhage syndrome. Acute respiratory distress syndrome (ARDS), which is a prominent feature of this manifestation, also can occur in the absence of documented bleeding. Pulmonary hemorrhage is one of the major causes of death in leptospirosis. In animal studies, Spichler et al[33] showed that leptospires appear to prefer organs such as the kidney or liver over the lungs. A morphologic study, under light microscopy, of the lungs of leptospirosis patients revealed edema of the intra-alveolar septa.[12] Mild to moderate inflammatory infiltrate was found, with a predominance of macrophages, amid lymphocytes and plasmocytes. In addition, endothelial tumefaction was seen, and some patients presented alveolar hemorrhage. Leptospiral antigen also was detected as positive granular material on the luminal surface of the endothelium and in the cytoplasm of the endothelial cells of septal capillaries, as well as in the filamentous form, attached to the endothelium of the septal capillaries.[12,34] In another animal study, Nally et al[35] used immunofluorescence staining to show that deposition of immunoglobulin can be granular (classic immune deposits as seen in certain renal diseases), or linear (as occurs in other renal diseases and Goodpasture's syndrome). Granular deposits are visible using immunofluorescence, electron microscopy, and sometimes even light microscopy. Linear deposits are seen through immunofluorescence, although not typically under electron microscopy. The pathogenesis of the lung disease in this experimental system best fits with a model of linear deposition of immunoglobulin and complement as occurs in Goodpasture's syndrome or antiglomerular basement membrane disease. The inflammatory infiltrate of monocytes and polymorphonuclear cells observed in thickened alveolar septa included some cells in which leptospiral antigen was shown by using immunohistochemistry. There are several possibilities to explain the presence of inflammatory cells observed in the alveolar septum: antigenic leptospiral debris found within the alveolar septum might reflect the clearance of intact spirochetes by inflammatory cells, endothelial damage evidenced by the blebbing formation of endothelial cells seen under electron microscopy might have drawn an inflammatory response, or, finally, complement activation evidenced by the detection of C3 might have caused the inflammation.[35]

In human patients, leptospirosis has many presentations, including the severe pulmonary form (ARDS), which is characterized by impairment of the alveolar-capillary barrier. It has been reported that pulmonary edema clearance is greatly affected by active sodium transport out of the alveoli rather than by reversal of the Starling forces. Andrade et al[21] showed that leptospirosis infection decreases α-ENaC protein expression in lung membranes of hamsters infected with leptospirosis. The investigators also found that basolateral protein expression

of the Na-K-2Cl cotransporter NKCC1 was upregulated, as well as that aquaporin 5 and α-Na-K–adenosine triphosphatase protein expression were unchanged, in the lung tissue of hamsters infected with leptospirosis.[21] These results show the effects of leptospirosis on alveolar ion transporters, which have been shown to play a vital role in the maintenance of alveolar fluid. Active transport by the α-Na-K–adenosine triphosphatase pump generates an osmotic driving force favorable to the entrance of sodium via α-ENaC. There is therefore continuous transport of sodium from the lumen into the interstitial space. Despite the presence of aquaporin 5, the osmotic gradient between the lumen and the interstitial space promotes the movement of water via the paracellular pathway. Volume is regulated primarily by electroneutral cotransporters such as NKCC1, which is found in virtually all cells and mediates coupled influx of sodium, potassium, and chlorine. We can therefore hypothesize that leptospirosis induces a decrease in alveolar clearance by decreasing α-ENaC protein abundance, which reduces the transport of sodium from the lumen into the interstitial space, as well as decreases the movement of water from the lumen into the interstitial space, thereby lowering the osmotic gradient. The cell shrinkage induced by this mechanism can stimulate the NKCC1 protein in the basolateral membrane. In turn, NKCC1 mediates the coupled influx of sodium, potassium, and chlorine into the epithelial cells. The decreased influx of sodium from the lumen into the cells (induced by the lower levels of α-ENaC), together with the increased influx of sodium from the interstitial space into the cells (induced by the higher levels of NKCC1), can block the net influx of sodium and water from the alveoli (Fig. 1). Impaired pulmonary fluid clearance resulting from down-regulated α-ENaC expression, as well as the potential derangements related to increased NKCC1 expression, might have significant deleterious effects in the context of increased pulmonary permeability such as that observed in ARDS.

The administration of methylprednisolone has been used as a means of slowing or halting the progression of pulmonary leptospirosis. In a small sample of patients, the overall mortality rate decreased in the group receiving methylprednisolone.[36] It remains unclear whether corticosteroids provide any real benefit to patients with pulmonary leptospirosis.

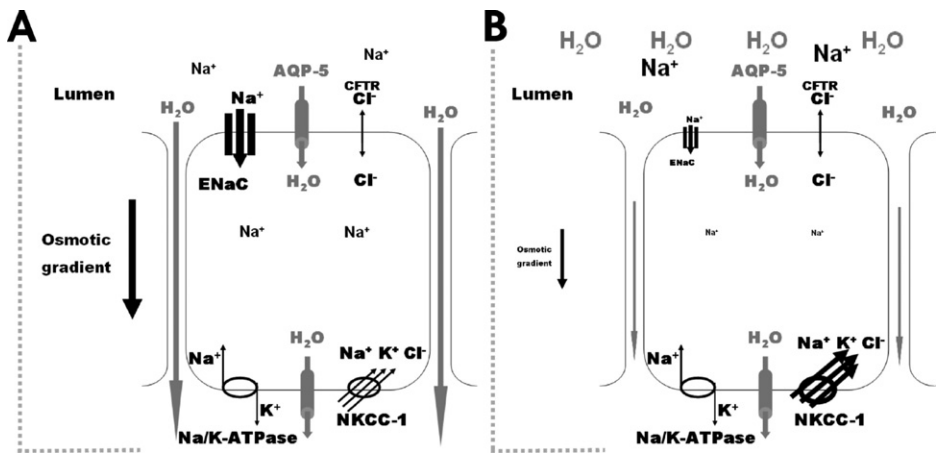

Figure 1. (A) Normal: active transport by the Na-K-adenosine triphosphatase (ATPase) pump generates an osmotic driving force that favors the entrance of sodium via α-ENaC. There is therefore continuous transport of sodium from the lumen into the interstice. Despite the presence of aquaporin 5 (AQP-5), the osmotic gradient between the lumen and the interstice promotes the movement of water via the paracellular pathway. Cotransport of NKCC1 regulates cellular volume. (B) In leptospirosis the decreased influx of sodium from the lumen into the cells (induced by the lower levels of α-ENaC protein), together with the increased influx of sodium from the interstitial space into the cells (induced by the higher levels of NKCC1), can block the net influx of sodium and water from the alveoli. (A and B) Note that text size and arrow size are related to the degree of protein expression, level of sodium, osmotic gradient, and so forth. Reprinted and used with permission from Andrade et al, Am J Renal Physiol 292:F586-F592, 2007.[21]

LEPTOSPIROSIS IN THE INTENSIVE CARE UNIT

Pulmonary edema/hemorrhage leading to ARDS constitutes the most severe manifestation of lung injury in leptospirosis.

The ability of the lungs to resolve edema is crucial for restoring lung function and is known to be impaired in patients with ARDS.[37,38] A strong association between AKI and ARDS has been shown consistently. It also has been shown that respiratory and renal failure are associated independently with mortality.[39] Weil's disease manifests as severe lung injury (diffusive alveolar hemorrhage, pulmonary edema, ARDS, or a combination of these features) accompanied by AKI and therefore can be highly lethal.[20,34]

Leptospirosis is a model of sepsis. In a series published by Marotto et al,[20] the initial hemodynamic profile of a group of 12 patients was as follows: high cardiac index (4.71 ± 1.41 L/min/m^2), normal pulmonary capillary wedge pressure (10 ± 5 mm Hg), and a low mean systemic vascular resistance (1,393 ± 882 dyne/s/cm^5). This hemodynamic profile is consistent with a diagnosis of sepsis. In the intensive care unit of the Emílio Ribas Institute of Infectology, the mortality rate among patients with leptospirosis and ARDS (on mechanical ventilation) and AKI (on dialysis) was 55% from 1994 to 1997 and 43% from 1998 to 2001.[40] Recent evidence suggests that dialysis dosage affects outcomes in critically ill patients with sepsis-induced AKI.[41] Based on these studies, the effects of dialysis dosage were evaluated in the severe form of Weil's disease: patients with leptospirosis and ARDS (on mechanical ventilation) and AKI (on dialysis).[40] The investigators found that the prompt initiation of dialysis, together with daily dialysis sessions, reduced the mortality rate to 16.7%, compared with 66.7% among the patients who performed hemodialysis on alternate days. Based on these results, they concluded that alternate-day hemodialysis should no longer be consider appropriate for critically ill patients with Weil's disease.

Because of the risk of pulmonary hemorrhage, it is recommended that daily net fluid intake be maintained at low levels in patients with leptospirosis. The ARDS Clinical Trials Network study showed that a conservative fluid management protocol aimed at achieving lower central venous pressure or lower pulmonary artery occlusion pressure resulted in a greater reduction in the net intake without an increase in adverse events, as compared with a liberal fluid management protocol aimed at achieving higher intravascular volume and cardiac filling pressures.[42] The conservative strategy improved lung function, shortening the duration of mechanical ventilation and intensive care unit stay without increasing nonpulmonary organ failure. Theses results lend credence to the idea that a conservative strategy of fluid management should be used in patients with acute lung injury.[42] It also has been shown that leptospirosis has a profound influence on the sodium transport capacity of alveolar epithelial cells, inducing a decrease in alveolar clearance.[21]

During mechanical ventilation, it also is recommended that lung-protective strategies based on low tidal volumes (6 mL/kg) be used to guarantee lower plateau pressures. High positive end-expiratory pressures after recruitment maneuvers, used to ensure alveolar stabilization and recovery of gas exchange, have been associated with decreased mortality rates in this critical condition.[43,44]

TREATMENT

Antibiotic treatment is efficient in the early and late/severe phases of the disease. A recent study in leptospirosis-infected hamsters used immunohistochemistry to show that infected animals presented high amounts of detectable leptospiral antigens in target tissues and decreased renal expression of NHE3 and NKCC2. Early and late ampicillin treatment was associated with minimal or no detection of leptospiral antigens and with restoring normal expression of NHE3 and NKCC2.[33]

Severe leptospirosis is treated with intravenous penicillin (1,500,000 U every 6 h). Intravenous ceftriaxone (1 g once daily) or cefotaxime (1 g every 6 h) present efficacy equivalent to that of penicillin.[45,46] Treatment must be maintained for 7 days. Although Jarisch-Herxheimer reactions during initiation of an antibiotic can occur, they are less common in leptospirosis than in other spirochetal infec-

tions. Azithromycin has been found to be effective in the treatment of leptospirosis in patients who were ambulatory and did not present involvement of vital organs, therefore representing an effective alternative to penicillin in the treatment of less severe cases of leptospirosis.[47] Doxycycline also can be effective in these patients.[48,49]

PREVENTION AND VACCINES

There are few preventive measures for leptospirosis. Doxycycline prophylaxis (200 mg/wk) does not prevent leptospiral infection in an endemic area, although it has a significant protective effect in reducing morbidity and mortality during outbreaks.[50] Another preventive measure would be the use of protective clothing, such as boots, although that it is not feasible in most situations. Control of *Leptospira* reservoirs is difficult given the complex ecologic interactions among domestic reservoirs, wild reservoirs, and environmental transmission sources.[1] An effective vaccine for leptospirosis has yet to be developed. To date, all leptospirosis vaccines have been produced using killed bacteria and provoke unacceptable side effects, as well as presenting only short-term efficacy.[3]

GENOME AND FUTURE PERSPECTIVES

The complete genome sequences of some species of *Leptospira* have been described recently by Chinese and Brazilian researchers.[51,52] Ren et al[51] sequenced the genome of *L interrogans* serovar *Lai*, strain 56601, which is responsible for infections in rural areas of China. Nascimento et al[52] sequenced the genome of *L interrogans* serovar *copenhageni*, strain Fiocruz L1-130, which causes urban outbreaks in Brazil.

The genome of *Leptospira* consists of 2 circular chromosomes and is highly conserved between the 2 serovars.[51,52] The discovery of bacterial genomes is one of the most important revolutions in the field of infectious diseases. The sequencing of this genome creates an invaluable source of information for researchers, and its applications include the development of specific culture media, identification of antibiotic-resistance mechanisms, identification of virulence factors, and clarification of host–pathogen interaction mechanisms, as well as the development of monoclonal antibodies and vaccines.[53,54]

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