

Sustained Tubulointerstitial Inflammation in Kidney with Severe Leptospirosis

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Abstract

Leptospirosis is frequently associated with acute kidney injury. Some survivors are known to progress to chronic kidney disease due to sustained tubulointerstitial inflammation. We present a case of severe leptospirosis with acute renal failure. Although antibiotic therapy resolved the infection, moderate renal dysfunction remained. A renal biopsy demonstrated marked inflammatory infiltration in the tubules and interstitium. Many of the inflammatory cells were CD68-positive monocytes/macrophages, predominantly M1 phenotype. An intermediate dose of oral corticosteroids normalized the patient's serum creatinine levels. We suggest that corticosteroid therapy may be a therapeutic option for some patients with sustained tubulointerstitial nephritis who survive severe leptospirosis.

Key words: leptospirosis, acute kidney injury, macrophages

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Introduction

Leptospirosis is a common zoonotic infection in tropical regions, but it has been identified as an emerging infectious disease even in developed countries (1). The clinical presentations vary from mild flu-like illness to severe septicemia with acute renal failure, hepatic necrosis, pulmonary hemorrhaging, and/or cardiovascular collapse (2). Renal involvement is frequently seen in severe instances of the disease. Such involvement is mainly characterized by pathogen-induced acute tubulointerstitial nephritis as well as acute tubular necrosis caused by hemodynamic alterations and the immune response (3). These pathological alterations have been found in autopsy-based case reports and case series. Even though some patients who survive severe leptospirosis have prolonged renal dysfunction, little evidence has been presented regarding the renal pathology after resolution of the infection in survivors with renal impairment.

Acute kidney injury has been increasingly considered a

major cause of chronic kidney disease (CKD) and end-stage renal disease. Survivors of severe leptospirosis were previously assumed to recover their renal function. However, recent reports have suggested that a significant proportion of these patients have incomplete renal recovery and progress to CKD (4). Sustained renal inflammation after resolution of the infection should be attributed to this progression. In particular, persistent monocyte/macrophage (M1 but not M2 phenotype) infiltration probably has an adverse effect, preventing renal histological and functional recovery. It is worth considering a renal biopsy for such patients in order to predict their renal prognosis and establish therapeutic strategies, whenever possible.

In this report, we present a case of severe leptospirosis associated with oligouric renal failure. After successful antibiotic therapy, a renal biopsy revealed sustained tubulointerstitial nephritis. Immunohistochemistry demonstrated the accumulation of macrophages, predominantly M1 phenotype. Oral corticosteroids with tapering improved the patient's renal function completely. This case report suggests the possi-

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Table 1. Laboratory Data on Admission.

<i>Blood Count</i>		<i>Chemistry</i>		<i>Immunology</i>	
WBC	26,700 / μ L	TP	5.0 g/dL	IgG	908 mg/dL
Neu	87.4 %	Alb	1.8 g/dL	IgA	164 mg/dL
Lym	2.2 %	T-Bil	3.0 mg/dL	IgM	34 mg/dL
Eos	6.6 %	D-Bil	2.2 mg/dL	C3	86 mg/dL
Baso	0.0 %	AST	86 IU/L	C4	32.8 mg/dL
RBC	284 \times 10 ⁴ / μ L	ALT	24 IU/L	CH50	45 mg/dL
Hb	11.0 g/dL	AMY	2,512 IU/L	ANA	\leq 40 (-)
Ht	32.5 %	LDH	336 IU/L	RF	(-)
MCV	114.4 fL	ALP	469 IU/L	MPO-ANCA	<0.50 (-)
MCH	38.7 pg	γ GTP	105 IU/L	PR3-ANCA	<0.50 (-)
MCHC	33.8 %	UA	7.7 mg/dL	GBM Ab	<10 (-)
Plt	3.2 \times 10 ⁴ / μ L	Cr	4.55 mg/dL	<i>Coagulation</i>	
		BUN	73.6 mg/dL	PT	12.1 sec
		Na	134 mEq/L	APTT	38.4 sec
		K	4.7 mEq/L	Fib	629 mg/dL
		Cl	94 mEq/L	FDP	33.2 μ g/mL
		Ca	7.1 mg/dL	D-dimer	12.6 μ g/mL
		IP	7.8 mg/dL	<i>Infection</i>	
		T-chol	124 mg/dL	HBs Ag	(-)
		CK	284 mg/dL	HCV Ab	(-)
		CRP	43.5 mg/dL	HIV Ab	(-)
		HbA1c	5.7 %	Parvo IgM	(-)
		U-NAG	116.8 U/L	PCT	95.1 ng/mL
<i>Urinalysis</i>					
pH	6.0				
SG	1.010				
Pro	4+				
OB	3+				
Uro	\pm				
<i>Sediment</i>					
RBC	10-15 /HF				
WBC	5-10 /HF				
ETC	3-5 /HF				

SG: specific gravity, Pro: protein, OB: occult blood, Uro: urobilinogen, ETC: epithelial cells, U-NAG: urinary N-acetyl- β -D-glucosaminidase, Ab: antibody, PCT: procalcitonin

ble benefit of post-infectious corticosteroid therapy for some patients with leptospiral nephropathy in order to prevent the progression to CKD.

Case Report

A 63-year-old man presented with fever and general malaise followed by disturbance of consciousness and hypotension. At admission, laboratory testing showed acute renal failure, hepatic injury and disseminated intravascular coagulation (DIC), as shown in Table 1. The elevated procalcitonin (PCT) level of 95.1 ng/mL suggested the presence of a bacterial infection. However, imaging studies, including thoracoabdominal computed tomography (CT), did not reveal any findings indicating the origin of the infection, and no bacteria were detected by culture testing of blood, urine and cerebrospinal fluid samples. We started broad-spectrum antibiotic therapy with meropenem and vancomycin in addition to norepinephrine infusion and intravenous recombinant thrombomodulin, and then carried out intermittent hemodialysis therapy for oliguric renal failure. The peak values during the treatment were as follows: white blood cells 26,700/ μ L, hemoglobin 11.0 g/dL, platelets 28,000/ μ L, total-bilirubin 7.3 mg/dL, direct-bilirubin 5.3 mg/dL, blood urea nitrogen 68.7 mg/dL, creatinine 6.30 mg/dL and C-reactive protein (CRP) 43.5 mg/dL. These treatments resulted in improvement in his general condition and laboratory data, including platelet counts and CRP and bilirubin levels

(Fig. 1). After three weeks, the patient recovered his urine volume, and hemodialysis therapy withdrawn. On assessing paired serum samples obtained at admission and after four weeks, we found that the agglutinating antibody titers against *Leptospira interrogans* serovar *Hebdomadis* and serovar *Rachmati* had markedly increased to 1:160 and 1:320, respectively, leading to a diagnosis of leptospirosis (Table 2).

Despite an improvement in the patient's condition and most laboratory data, the renal impairment persisted, with an elevated serum creatinine level between 2.5 and 3.0 mg/dL. CT showed slight bilateral renal swelling. ⁶⁷Ga-citrate scintigraphy demonstrated prominent accumulation in the bilateral kidneys (Fig. 2). A renal biopsy revealed the renal histological alterations after resolution of leptospiral infection. Detached tubular epithelial cells from the basement membranes as well as regenerating tubules with accumulation of nuclei were observed. We also noted tubulitis lesions with marked mononuclear cell infiltration in the tubules and interstitium (Fig. 3a and b). No apparent changes were observed in the glomeruli. On Hematoxylin and Eosin (H&E) staining, these mononuclear cells were compatible with lymphocytes/monocytes (Fig. 3c and d). Eosinophils and plasma cells were scarcely seen among the mononuclear cells. These findings suggested persistent tubulointerstitial nephritis with regeneration after insult of acute tubular necrosis.

To clarify the characteristics of the renal inflammatory cells after resolution of the leptospiral infection, the immu-

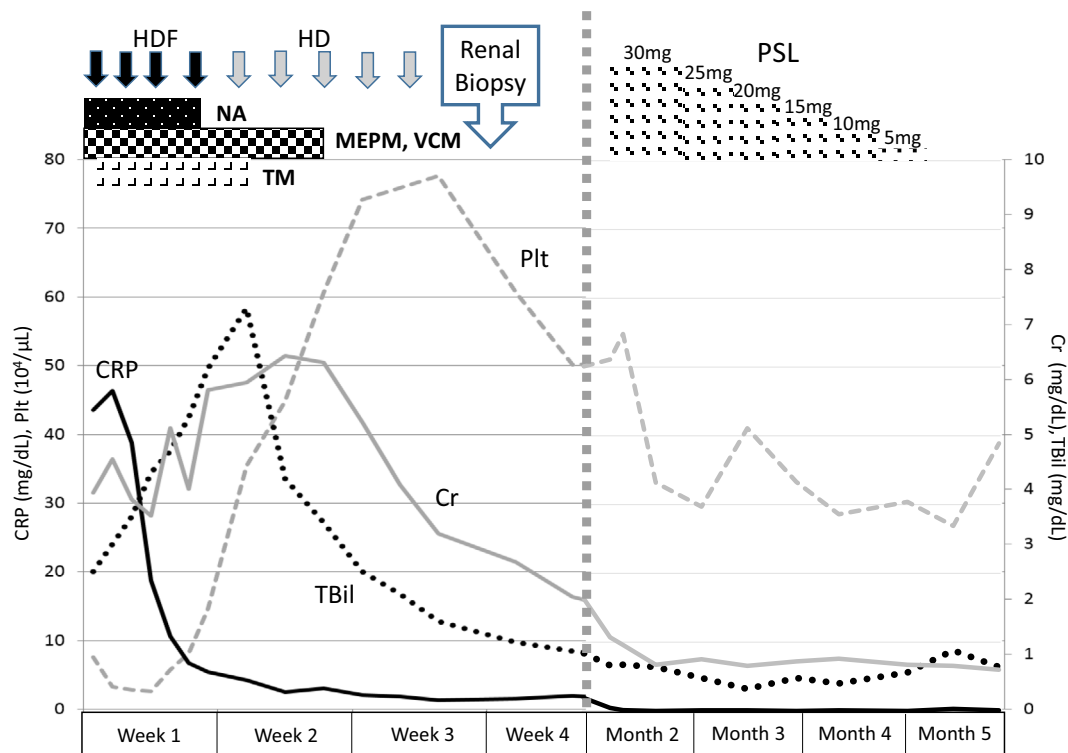


Figure 1. The clinical course of the case from admission to the renal biopsy. HDF: hemodiafiltration, HD: hemodialysis, NA: norepinephrine, MEPM: meropenem, VCM: vancomycin, TM: thrombomodulin, PSL: prednisone (with daily oral dosage)

Table 2. Results of Microscopic Agglutination Test from Paired Serum Samples at Admission and after Four Weeks.

The species or serovars of <i>Leptospira</i>	Antibody titer	
	At admission	Four weeks later
<i>Leptospira borgpetersenii</i> serovar <i>Castellonis</i>		<40
<i>L. borgpetersenii</i> serovar <i>Javanica</i>		<40
<i>L. borgpetersenii</i> serovar <i>Poi</i>	<10	80
<i>L. interrogans</i> serovar <i>Australis</i>		<40
<i>L. interrogans</i> serovar <i>Autumnalis</i>	<10	80
<i>L. interrogans</i> serovar <i>Bataviae</i>		<40
<i>L. interrogans</i> serovar <i>Canicola</i>		<40
<i>L. interrogans</i> serovar <i>Copenhageni</i>		<40
<i>L. interrogans</i> serovar <i>Hebdomadis</i>	10	160
<i>L. interrogans</i> serovar <i>Icterohaemorrhagiae</i>		<40
<i>L. interrogans</i> serovar <i>Kremastos</i>	20	160
<i>L. interrogans</i> serovar <i>Pomona</i>		<40
<i>L. interrogans</i> serovar <i>Pyrogenes</i>		<40
<i>L. interrogans</i> serovar <i>Rachmati</i>	<10	320
<i>L. kirschneri</i> serovar <i>Grippityphosa</i>		<40

nohistochemistry of the renal biopsy specimens was evaluated (see Supplementary Material). In the section containing marked inflammatory infiltration in the renal interstitium, immunohistochemistry demonstrated that some of the mononuclear cells were positive for CD68 antigen, which is a marker for monocytes/macrophages (Fig. 3e). Furthermore, the phenotype of the monocytes/macrophages was confirmed by immunohistochemistry to be HLA-DR (M1 antigen) and

CD163 (M2 antigen). HLA-DR-positive mononuclear cells representing M1 macrophages obviously predominated in the renal interstitium (Fig. 3f) compared with CD163-positive M2 macrophages, as shown on the serial sections under high magnification (Fig. 3g-i). Despite the prominent tubulointerstitial inflammation, no leptospiral antigens, including those for *L. interrogans* serovar *Hebdomadis* and serovar *Rachmati*, were stained on the sections with leptospiral

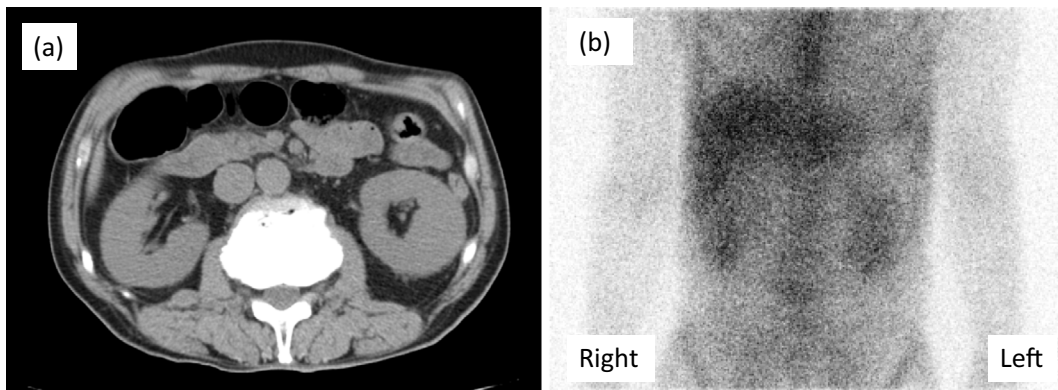


Figure 2. Renal imaging. a: Computed tomography showed slight bilateral renal swelling. b: ^{67}Ga -citrate scintigraphy demonstrated prominent tracer accumulation in the bilateral kidneys.

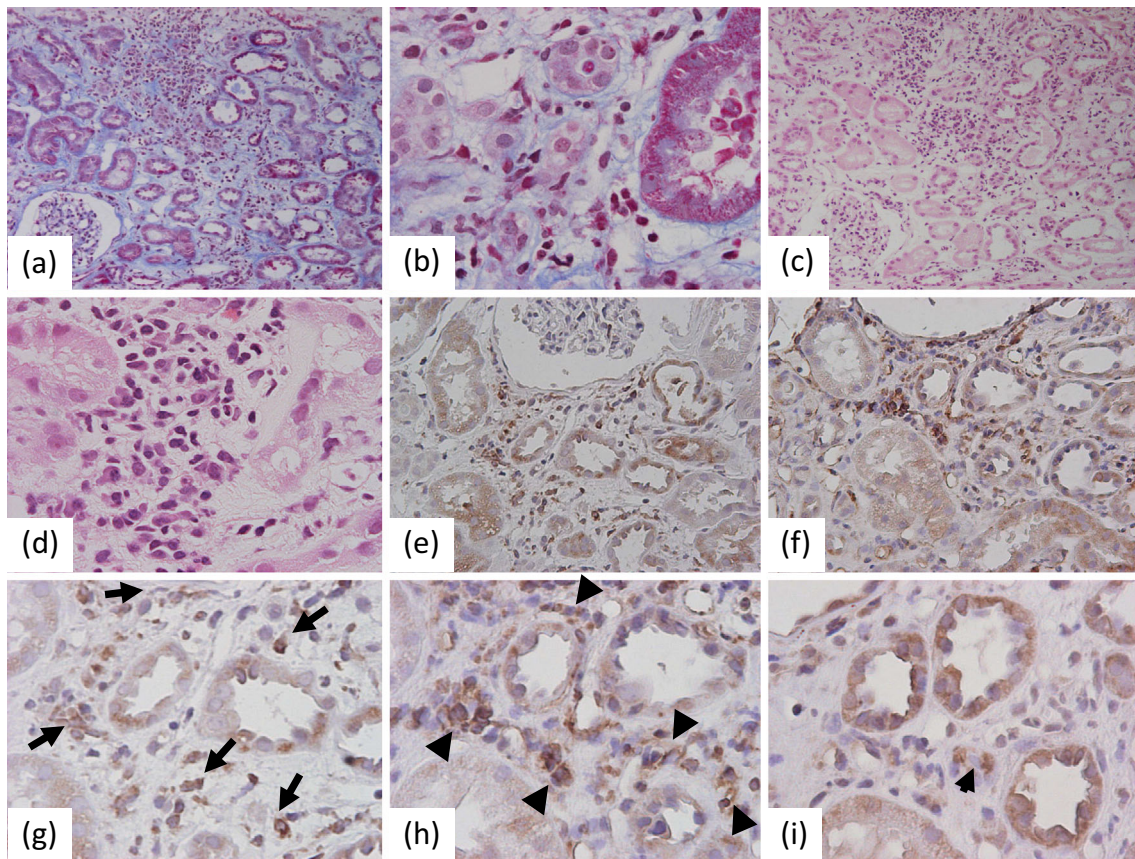


Figure 3. Renal histology of biopsy specimens. a, b: Marked mononuclear cell infiltration in the tubules and interstitium on Masson's trichrome stained section. c, d: Hematoxylin and Eosin staining showed that almost all of the mononuclear cells were lymphocytes/monocytes. 100 \times and 400 \times original magnification, respectively. e, g: Immunohistochemistry for CD68 revealed that many of the inflammatory cells were monocytes/macrophages (long arrows). Further study demonstrated that these macrophages were predominantly HLA-DR-positive M1 phenotype (f, h; arrow heads) versus the CD163-positive M2 phenotype (i; short arrow) on serial sections. 200 \times and 400 \times original magnification.

serum antibodies (data not shown).

Since antibiotic therapy had resolved his infectious symptoms and increased the PCT levels, oral administration of intermediate-dose corticosteroids at 0.6 mg/kg body weight for sustained tubulointerstitial nephritis was started, with gradual tapering. Four weeks after starting the treatment, his

serum creatinine levels returned to the normal range (Fig. 1). There were no instances of re-elevated serum creatinine or recurrent infection.

Discussion

Renal involvement is a well-known complication in leptospiral infection. The major renal histological change in leptospirosis is tubulointerstitial nephritis, which is even observed in patients without renal dysfunction. Tubular necrosis is also common, indicative of acute kidney injury (AKI). Patients with leptospiral AKI usually recover their renal function to the normal range. However, in a previous study of 58 patients with leptospiral AKI, only 12% of survivors had normal creatinine levels at discharge, and the same 12% had still not achieved normal creatinine levels after 90 days (5). Another study followed 22 patients with leptospiral AKI for more than 6 months. The patients with incomplete renal recovery tended to be older and achieved their maximal GFR later than those who recovered well (6). Furthermore, a recent report suggested that a small percentage of patients who survived acute leptospirosis had a persistently abnormal renal function consistent with early-stage CKD over a three-year follow-up period. Sustained tubulointerstitial lymphocyte infiltration might be an important factor influencing the progression to CKD (4).

How can renal inflammation persist after the elimination of leptospiral antigens? The pathogenesis of leptospirosis can be divided into direct effects by the organism and host immune response to the infection. Following acute infection, direct injury from leptospira antigen is caused by proteins present in the outer membrane of the leptospira (OMP). These proteins may activate Toll-like receptor (TLR)-dependent pathways, which leads to tubular injury via cytokines, and also activate the transforming growth factor- β /Smad-mediated fibrosis pathway (7, 8). However, in an animal experiment, acute tubulointerstitial nephritis complicated by leptospirosis was demonstrated even in TLR4 and TLR2 double-knockout mice (9), suggesting the pivotal role of a TLR-independent lymphocyte-activating mechanism. Along this line, the presence of far fewer leptospiral antigens in the lung than in the liver or kidney supports an indirect mechanism associated with the host's immune response to the infection. In ocular leptospirosis, the autoimmune response is believed to be the main pathogenesis, where a locally-produced antibody activates the complement cascade (1). Such an indirect immune response may be associated with the persistent renal inflammation seen in leptospirosis.

Renal histology from this case revealed moderate inflammatory infiltration in the tubules and interstitium, with concomitant tubular regeneration. Indeed, such tubulointerstitial lymphocyte infiltration seems to be compatible with the previous report on a renal biopsy in patients with leptospirosis (4), but tubulointerstitial inflammation induced by other causative agents such as antibiotics could not be excluded in this case. Interestingly, immunostaining of his renal specimen did not reveal any leptospiral antigens. Intact leptospira and its fragments have been observed throughout the tubular basement membrane in the acute infection period (10). Ad-

ditional immunohistochemistry demonstrated that many inflammatory cells were monocytes/macrophages, and the M1 phenotype dominated over the M2 phenotype. Monocytes/macrophages are generally activated by the TLR ligand and interferon gamma in response to bacterial infections, leading to an M1 phenotype. M1 macrophages may induce inflammatory cytokines and reactive oxygen species and promote anti-bacterial immune responses (11). In contrast to M1 macrophages, the M2 phenotype may act later in the anti-inflammatory response to promote healing and angiogenesis. The predominance of the M1 phenotype in renal specimens might reflect on-going active inflammation, possibly through persistent inflammatory mediators.

In the present case, the patient's renal function recovered to the normal range after the administration of oral corticosteroids for persistent renal inflammation. In a previous report, Kyle Minor et al. successfully treated a patient with severe leptospirosis exclusively with intravenous antibiotics and corticosteroids (2). The routine use of corticosteroid has not been well established without concurrent antibiotic therapy. Steroid therapy is usually administered to treat persistent tubulointerstitial nephritis after the removal of the causative drugs or infections. Follow-up without corticosteroids in such a setting could lead to slow improvement of the renal function, but immediate suppression of the renal inflammation and early recovery of the renal function are so crucial that persistent renal inflammation probably causes an increased risk of progression to CKD (12). Indeed, a few patients developing CKD after leptospiral infection have been shown to have persistent renal inflammatory infiltration (4). Furthermore, corticosteroids are suggested to inhibit M1 macrophage activation in favor of M2 macrophage differentiation, leading to kidney repair (11). Considering the tissue-repairing property of M2 macrophages, it may be better to start inhibition of macrophages with corticosteroids after the demonstration of M1 predominance.

Although the current investigation is single case report, the histochemical examination in our case and previous case series suggested that some patients who survive severe leptospirosis may have sustained renal M1 macrophage infiltration. Among patients with a persistently abnormal renal function after resolution of leptospiral infection, a renal biopsy should be considered to provide histological evidence of persistent tubulointerstitial nephritis without residual leptospiral antigens, and if present, oral corticosteroids may be a therapeutic option for preventing the progression to CKD.

Author's disclosure of potential Conflicts of Interest (COI).

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