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T. De Brito · L. F. Menezes · Dirce M. C. Lima · Silvia Lourenço · Ana Maria G. Silva · V. A. F. Alves

Immunohistochemical and in situ hybridization studies of the liver and kidney in human leptospirosis

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Abstract An in situ hybridization (ISH) assay for the detection of leptospiral DNA in tissues was described and its diagnostic and pathogenetic usefulness in combination with immunohistochemistry (IHC) was evaluated in formalin-fixed, paraffin-embedded liver and kidney samples from human fatal cases of leptospirosis. IHC assays with anti-E-cadherin antibodies assessed the liver-plate disarray frequently observed in leptospirosis. Immunohistochemistry detected leptospiral antigen (LAg) in macrophages, both in human liver and kidney. In guinea pigs, in addition to these findings, staining on cell membranes of hepatocytes and, occasionally, in apical membrane of kidney tubular cells was demonstrated. Positive ISH signal was observed chiefly in the nuclei of human hepatocytes and in the cytoplasm and nuclei of liver cells of experimentally infected guinea pigs. Loss of E-cadherin membrane expression is associated with liver-plate disarray. These findings were discussed in the contention that, in leptospirosis, cell membrane damage might be important for the pathogenesis of the disease. Finally, it was suggested that both IHC and/or ISH might be used for both diagnostic and research purposes.

T. De Brito (✉) · L. F. Menezes · D. M. C. Lima · S. Lourenço · A. M. G. Silva · V. A. F. Alves
Institute of Tropical Medicine,
University of Sao Paulo Medical School,
Sao Paulo, SP, Brazil
e-mail: thalesbr@usp.br
Tel.: +55-11-30667066
Fax: +55-11-30645132

T. De Brito · L. F. Menezes · D. M. C. Lima · S. Lourenço · A. M. G. Silva · V. A. F. Alves
Department of Pathology,
University of Sao Paulo Medical School,
Sao Paulo, SP, Brazil

V. A. F. Alves
Adolpho Lutz Institute, Sao Paulo Health Service,
Sao Paulo, Brazil

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Introduction

Leptospirosis is an acute septicemic febrile illness, caused by *Leptospira interrogans*, which affects humans or animals in all parts of the world. In humans, it is a zoonosis and its reservoirs are chronically infected carrier animals. There is a worldwide strong occupational association and a universal risk from rodent-carrier-mediated infection, especially prevalent in tropical countries where it occurs in endemic and epidemic bouts, chiefly during the rainy season [7, 14].

Leptospirosis has a biphasic clinical presentation, beginning with a septicemic phase followed by an immune phase with antibody production and urinary excretion of leptospire. In severe cases, leptospirosis is a multisystemic febrile illness, chiefly with hepatic, renal, and pulmonary involvement. The most severe form of leptospirosis is known as Weil's syndrome and can lead to fatality in a short time [18].

Microscopic agglutination test remains an excellent serological assay and yields information on the presumptive infecting serogroup. In fatal human cases [1] and in farm animals [13], diagnosis by immunohistochemistry (IHC) is specific and also offers clues to the understanding of the pathogenesis of the disease.

Morphology and antigenic structure as well as the genetic composition and organization of ribosomes and nucleic acids of leptospires were recently reviewed [14]. rRNA genes are very conserved throughout the bacterial kingdom and partial sequences have been analyzed in terms of oligonucleotide signatures. Mérien et al. [19] reported the development of a sensitive assay for leptospires DNA based upon amplification of oligonucleotide sequences of the *Leptospira* rrs (16S) gene [15]. Recently, the complete genomic sequence of a representative virulent serovar type (*Lai*) of *L. interrogans* was obtained by the

Chinese National Human Genome Center at Shanghai [28]. The genomic sequence of *L. interrogans*, serovar *Copenhageni*, was established by a Brazilian group [21, 22].

In the light of these results, this paper describes an in situ hybridization assay (ISH) for the detection of leptospire DNA in tissues. Liver and kidney lesions were chosen for analysis because, with vascular lesions, they form an important part of the systemic manifestations of leptospirosis. The diagnostic and chiefly the pathogenetic usefulness of this technique were evaluated and compared to immunohistochemistry in formalin-fixed, paraffin-embedded liver and kidney samples from human fatal cases of leptospirosis. In addition, E-cadherin expression was used as a marker of cell–cell junction integrity to assess the specificity and potential pathogenetic role of liver-plate disarray, a common histological finding in leptospirosis.

Materials and methods

Human samples

Ten patients, eight males and two females, ranging from 20- to 68 years old, were autopsied with a diagnosis of Weil's syndrome. Major clinical and laboratory findings are shown in Table 1. In brief, illness duration ranged from 3 to 15 days (average of 7.8 days) and all patients commenced suddenly with headache, malaise, muscle pains and, except for one, high fever. All of them developed marked jaundice, palpable enlarged liver, acute renal failure, and, with one exception, pulmonary and digestive tract hemorrhage with conjunctival suffusions. Epidemiological data showed that most patients had had contact with contaminated water, mainly from floods and sewage cleaning. Serology was performed in five patients. One of them had enzyme-linked immunosorbent assay (ELISA) IgM+seroagglutination 1/3,200 for *L. interrogans*, serovar, and serogroup *Cynopteri.* In three other patients, with 5 days of illness, serological tests were negative. In another patient, also with 5 days of illness, blood culture was positive for *L.interrogans* serovar *Butembo*, serogroup *Autumnalis*.

Kidney and liver samples were obtained from autopsies performed after 6 to 12 h of death (average 9 h). The tissues were fixed in formalin, embedded in paraffin, and routinely stained with hematoxylin eosin. The kidney and liver samples of eight non-leptospirotic patients were obtained from autopsies performed within the same post-mortem interval; three normal liver biopsies obtained from liver

Table 1 Leptospirosis: clinical and laboratory data

Case no./ sex/age (years)	Clinical and epidemiological information	Illness duration (days)	Laboratory data related to leptospirosis
1/f/54	Contact with contaminated water (floods); fever, headache, conjunctival suffusion, muscular pain, acute renal failure, hemorrhagic sputum, jaundice, dehydration	11	ELISA IgM+, seroagglutination 1/3,200 (*L. interrogans* serovar and serogroup *Cynopteri*)
2/m/58	Contact with contaminated water (floods); fever, jaundice, muscular pain, acute renal failure, dehydration, pulmonary hemorrhage	5	Serological tests for leptospirosis, negative
3/m/59	Contact with contaminated water (fall into a well); muscular pain, acute renal failure, jaundice, dehydration	3	Blood culture positive for *L. interrogans* serovar *Butembo*, serogroup *Autumnalis*
4/m/53	Contact with contaminated water (floods); fever, jaundice, muscular pain, acute renal failure, dehydration, hemorrhagic syndrome (pulmonary and digestive tract)	15	None available
5/m/46	Contact with contaminated water (floods); fever, jaundice, muscular pain, dyspnea and hemorrhagic sputum	5	None available
6/m/68	Fever for the last 5 days, muscular pain, jaundice, acute renal failure	7	Seroagglutination negative, ELISA IgM positive
7/m/27	Contact with sewage and rats; fever for the last 5 days, jaundice, muscular pain, acute renal failure, hemorrhagic syndrome (pulmonary and digestive tract)	5	Seroagglutination negative, ELISA IgM positive
8/m/51	Fever for the last 7 days, marked jaundice and muscular pain for the last two days, anemia, leukocytosis, thrombocytopenia and renal failure; patient was HIV+	7	None available
9/m/20	Fever, muscular pain for the last 5 days, jaundice for the last 4 days, bipalpebral edema and acute renal failure, pulmonary hemorrhage, abdominal pain and vomits, low platelets count	5	None available
10/f/67	Contact with rats and contaminated water; fever and muscular pain for the last 8 days, jaundice for the last 6 days and conjunctival hemorrhage, dyspnea and hemorrhagic sputum, acute renal failure	8	None available

transplant donors were used as negative controls. The samples were processed as described above.

Guinea pig samples

Fifteen guinea pigs were infected with a virulent culture of *L. interrogans*, serovar *Copenhageni*. The animals developed the disease 5 days after inoculation. Liver and kidney samples were obtained and processed as described above. The tissues were compared to the human samples and used as positive controls. Liver and kidney samples of nine non-infected guinea pigs were used as negative controls.

Immunohistochemistry assay

IHC studies were performed to assess the presence of leptospiral antigen/s (LAg) and abnormalities in cell–cell junctions. Two primary sera were used for the detection of LAg. One serum was obtained by hyper-immunizing the sheep with sequential intravenous doses of 1, 2, 3, and 4 ml of *L. interrogans*, serogroup *Icterohaemorrhagiae*. The leptospires were obtained from 7 to 10 days of cultures grown at 28°C in Ellinghausen–McCullough–Johnson–Harris medium (DIFCO). Seven days after the last inoculation, an 80-ml blood sample was drawn. IgG was obtained by octanoic acid fractionation, purified, and used in a 1:30,000 concentration in 1% bovine serum albumin (BSA) in phosphate-buffered saline (PBS). The second immune serum was raised in rabbits according to a previously described standard procedure [12].

Anti-E-cadherin monoclonal antibody was employed to assess cell–cell attachment status in liver. These studies were restricted to five leptospirotic and five non-leptospirotic patient autopsies and three liver transplant donor biopsies. All autopsies were performed within 6 h post-mortem and showed well-preserved histopathological structures.

Kidney and liver 3-μm sections were analyzed using EnVision (Dako, USA)-based immunohistochemistry methods. Antigen retrieval protocol, primary antibodies clones, sources, and concentrations are summarized in Table 2. Staining was completed with the chromogen 3,3′ diaminobenzidin. All specimens were then lightly counterstained with hematoxylin.

The presence of nonspecific staining with the LAg sheep antibody was assessed in experiments with pre-cleared sera. In brief, 1-ml aliquots of anti- *L. interrogans* antibody was absorbed at 4°C, overnight, with 100 mg of normal human liver and 30 mg of normal human kidney powder, and used for immunohistochemistry in tissues not subjected to antigen retrieval. In this procedure, the primary sheep antibody was diluted at 1:40,000 in 1% BSA in PBS. Reactions in which primary antibody was replaced with saline or normal human serum were also performed as negative controls.

In situ hybridization

Two oligonucleotide probes were synthesized to detect leptospiral nucleic acids. Probe one (5′-CAA GTC AAG CGG AGT AGC AAT ACT CAG CGG CGA ACG GGT G-3′, Eurogentec S/A) is a sense fluorescein-labeled probe complementary to sequences A and C used for polymerase chain reaction (PCR) assays described by Mérien et al. [19].

Probe two (5′-GAT CTG AAC TTT ATA GAT TGA TTT CTA AAA TGT-3′, Invitrogen, USA) was designed based on the published genomic sequence of *L. interrogans*, serovar *Lai*, serogroup *Icterohaemorrhagiae*. Genes coding for small proteins of unknown function, conserved in *Leptospira* but with low identity levels in vertebrates, were chosen as candidates for probe generation. Thirty to thirty-three base sense cDNA probes were designed using PRIMER3 [25] with default conditions. The probes thus obtained were tested for nonspecific human complementary sequences using short-sequence nucleotide–nucleotide Blast (http://www.nebi.nlm.nih.gov/BLAST); those with least similarity were also checked against hepatitis B virus genome to minimize the chances of cross-reaction. Using this approach, a sequence also present in *L. interrogans*, serovar *Copenhageni*, was chosen from the sequence AEO11585.1:119–250 and used for fluorescein-labeled cDNA synthesis. Both probes recognize sequences conserved in several species of leptospires, both pathogenic and saprophites. Probe one has 45% identity (a sequence of 18 nucleotides) and probe two 57% (sequence of 19 nucleotides) with the human genome.

A negative control probe (scrambled probe 5′-CAG AAC GCG AGA CGT TAG ACC TCA ATG ACG GAG GGC AGG T-3′, Invitrogen) was obtained from probe one using a scrambling algorithm developed by T. Schubert (http://www.personal.uni-jena.de/~sth/scramble.html). To reduce the possibility of cross-reactions with other genes, the scrambled probe was compared to genomic banks of

Table 2 Immunohistochemical protocols

Primary serum	Clone	Source	Antigen retrieval	Primary serum titer	Secondary antibody
L intrrogans (sheep polyclonal)	–	See text	Steamer in citrate, pH 6.0	1:30,000	EnVision peroxidase (DAKO code K1490)
L interrogans (rabbit polyclonal)	–	[12]	Steamer in citrate, pH 6.0	1:5,000	EnVision peroxidase (DAKO code K1490)
E-cadherin (mouse monoclonal)	Clone 36B5	Novocastra Lab, Newcastle, UK	Steamer in in TRS (DAKO code S1699)	1:25 (autopsies) 1:50 (liver biopsies)	EnVision peroxidase (DAKO code K4001)

various organisms using the BLAST program (http://www.ncbi.nih.gov/BLAST) showing no significant similarities.

For ISH, 3-μm sections were deparaffinized, rehydrated through a graded series of ethanol, and washed with water treated with 0.1% diethylpyrocarbonate (DEPC) for 5 min. The sections were incubated with 25 μg/ml of Proteinase K (Sigma, St Louis, MO, USA) in PBS for 10 min at 37°C and then permeabilized with 2% Triton in Tris–HCl, 10 mM, at room temperature. The sections were rinsed with DEPC water for 10 min and incubated at 95°C for 7 min in hybridization buffer (50% dextran sulphate, 20× SSC, 50× Denhart's, 10 mg/ml of salmon sperm DNA, 10 mg/ml of yeast tRNA, 1 mg/ml of Poly A–Poly C, 100 mg/ml of inorganic pyrophosphate in 1 M Tris buffer, pH 7.4, deionized formamide, and DEPC). The sections were then incubated overnight at 55°C, with the corresponding probe, at a concentration of 0.340 μg/μl in hybridization buffer. After incubation, the sections were washed in Tris–HCl, pH 7.4, at 37°C for 10 min and further washed with water, five times for 5 min.The slides containing the tissue sections were finally air dried and mounted with Vecta-Shield and the reactions were visualized under epi-fluorescence microscope.

As an alternative, after proteinase K 12.5 mg/ml during 10 min at 37°C, the probes were incubated overnight at 42°C in a different hybridization buffer (50% deionized formamide, 10% dextran sulphate, 2× SSC, and 0.4 mg/ml salmon sperm DNA).

Results

The clinico-epidemiological data of the selected patients were highly suggestive of leptospirosis. The illness was usually of short duration and this, associated with delayed clinical diagnosis, contributed to the lack of some important confirmatory laboratory tests. Autopsies showed classical findings described in patients dying of Weil's syndrome [5, 8, 24], including acute myositis with hyalin change of necrotic muscular fibers affecting chiefly calf musculature; pulmonary and/or digestive hemorrhage; focal myocarditis; and liver pathology manifested chiefly by cholestatic hepatitis with complete or partial liver-plate

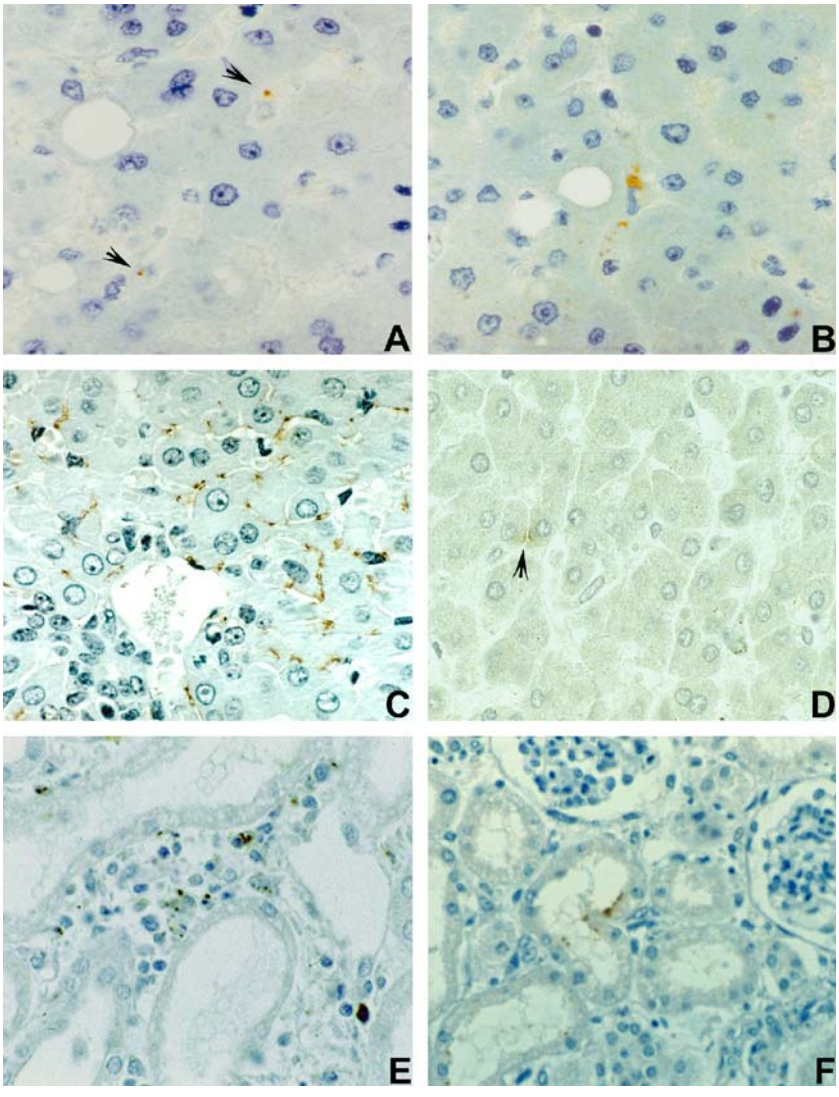

Fig. 1 **a** Liver in human fatal leptospirosis. LAg deposits visualized as small dots in the cytoplasm of Kupffer cells (*arrows*). There are isolated hepatocytes showing fatty change. IHC and light hematoxylin counterstain, ×400. **b** Liver in human fatal leptospirosis. LAg deposit in the cytoplasm of a Kupffer cell. Focal fatty change is also present. IHC and hematoxylin, ×400. **c** Experimental leptospirosis of the guinea pig. LAg detected on the liver cell membrane. IHC and hematoxylin, ×400. **d** Human fatal leptospirosis. Faint LAg deposit on the cell membrane of two adjacent liver cells (*arrow*). There is liver-plate disarray. IHC and hematoxylin, ×400. **e** Kidney in human fatal leptospirosis. LAg is present in the cytoplasm of monocytes of the kidney mixed interstitial inflammatory infiltrate. Tubules dilated with flat epithelial lining (acute tubular necrosis), ×400. **f** Kidney in human fatal leptospirosis. Focal LAg deposit on the luminal side of proximal tubule cells. Reactive nonspecific mesangioproliferative glomerulitis is also present. IHC and hematoxylin, ×300

disarray, occasional mitotic figures, and binucleation of hepatocytes; portal spaces' edema with acute and chronic inflammation; and proliferation of Kupffer cells along the sinusoidal lining [5]. Renal pathology showed focal interstitial nephritis, with lymphocytes, macrophages, plasma cells, and occasionally few eosinophils. Interstitial edema was observed in areas of tubular necrosis. In all cases, mild focal nonspecific reactive mesangio-proliferative glomerulonephritis was seen.

IHC analysis showed similar results with both immune sera. In addition, the experiments with pre-cleared sera confirmed the staining pattern. In nine of the ten patients, LAg was detected usually as dots, rarely as deposits in the cytoplasm of isolated macrophages which are part of the mixed portal inflammatory infiltrate, and more frequently in the cytoplasm of macrophages along the sinusoidal lining (Kupffer cells and circulating macrophages) (Fig. 1a,b). LAg expression was not observed on hepatocytes' membrane, except for one case in which a faint focal LAg deposit was detected on the cell membrane of two adjacent hepatocytes (Fig. 1d). In the kidney, LAg deposits were observed also in the cytoplasm of macrophages associated with the focal interstitial infiltrate (Fig. 1e). LAg deposits were seldom seen on the luminal side of tubular cells (Fig. 1f).

Diseased guinea pigs showed areas of liver-plate disarray and linear deposits of LAg on the hepatocyte membrane (Fig. 1c) as well as granular deposits in macrophages detected in portal spaces and Kupffer cells. In the kidney, the LAg deposits were observed in macrophages of the interstitial infiltrate. Linear deposits of LAg were occasionally seen on the luminal side of tubular cells.

Expression of E-cadherin was prominent on the cell membrane of hepatocytes both in liver transplant donor biopsies and in autopsy control specimens (Fig. 2a). In leptospirotic patients, expression of E-cadherin in liver cells was irregular. In areas of liver-plate disarray, hepatocytes without any expression of E-cadherin were present adjacent to hepatocytes with partial presence of E-cadherin on the cell membrane (Fig. 2b). In hepatic areas without disarray, the E-cadherin expression was similar to the control livers.

In situ hybridization staining revealed similar results with both probes and protocols. In nine patients, signal was observed in a few hepatocytes as an apple-green round or ring-like structure in the nuclei of liver cells (Fig. 2c). Only one patient (Table 1, case 9), in whom frequent mitotic figures could be detected in the liver, showed both cytoplasmic and nuclear signal (Fig. 2d). No convincing signal was seen in cells of the portal inflammatory infiltrate. Definite nuclear ISH signal was not observed in kidney tubular cells and was absent in macrophages of the interstitial focal inflammatory infiltrate.

The liver of 8 of 15 infected guinea pigs exhibited nuclear signal similar to that seen in human cases. In two of the eight guinea pigs, a small round cytoplasmic signal was also detected in the liver cells. No convincing signal was observed in the kidney. Human and guinea pig negative controls displayed no leptospiral ISH signals.

Discussion

Leptospirosis is an acute septicemic disease involving several organs. The demonstration by immunohistochemistry of LAg in cells of the inflammatory infiltrate both in human liver and kidney is expected as a response to circulating antigen/s. In the experimental model, LAg is

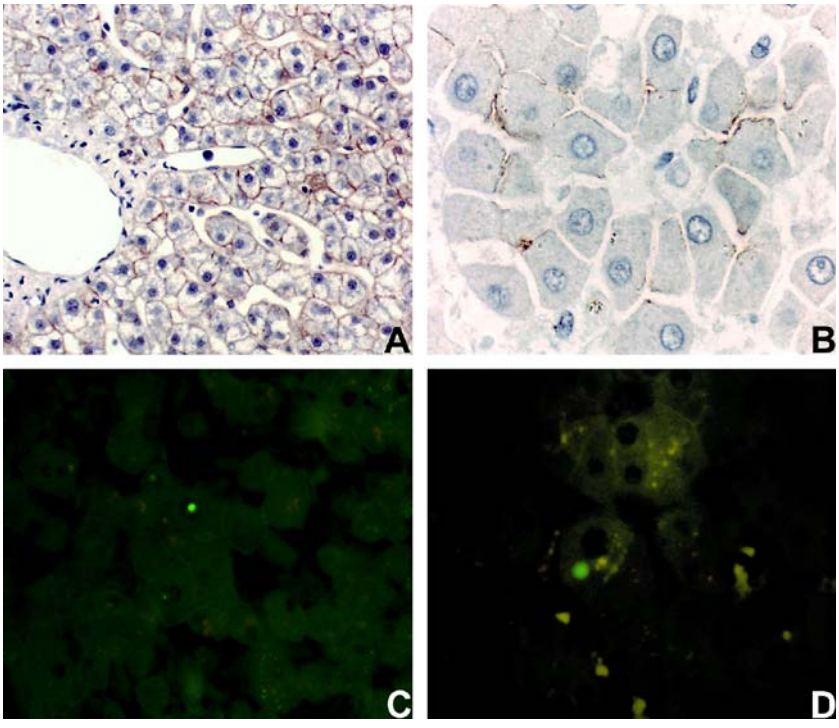

Fig. 2 **a** Control (non-leptospirotic) human liver showing normal E-cadherin expression over the cell membrane. IHC and hematoxylin, ×200. **b** Human fatal leptospirosis. Liver shows liver-plate disarray and irregular expression of E-cadherin. There are hepatocytes with no E-cadherin expression adjacent with others showing partial membrane E-cadherin expression. IHC and hematoxylin, ×400. **c** Human fatal leptospirosis. In situ hybridization (*ISH*). Hepatocyte showing nuclear signal. Fluorescent in situ hybridization (*FISH*), ×300. **d** Human fatal leptospirosis. Cytoplasmic signal close to the nucleus of a hepatocyte. FISH, ×400

demonstrated not only phagocytized by macrophages but also adhering to hepatocyte membrane, particularly at the zone III of the liver lobule where liver-plate disarray is usually more pronounced [2, 3, 20]. Adhesion of leptospires and/or their degradation products to cell and even to basement membranes and extracellular matrix has been described [2, 3, 14]. In our previous studies, immunoelectron microscopy in the guinea pig model of leptospirosis had revealed gold-labeled LAg close to cell membranes of hepatocytes, kidney tubular cells, and endothelial cells of the interstitial capillaries. The antigen/s were afterward internalized by hepatocytes and renal tubular cells and eventually found in lysosomes within damaged cells [12]. The affinity of leptospires for cell and basal membranes is directly related to its virulence. It is interesting that a fibronectin-binding protein expressed on the surface of virulent *L. interrogans*, serovar *Icterohaemorrhagiae*, is believed to be important for adhesion and invasion of leptospires [6].

In humans, LAg deposits are usually not found on the membrane of hepatocytes and can seldom be detected at the luminal side of renal tubular cells. The difference in expression pattern between the human disease and the guinea pig model may be attributed to several reasons: (1) in the experimental model, the number of injected leptospires could be much larger than in average human infection, leading to higher detection rate in the animals; (2) as mentioned earlier, LAg is initially localized to the cell membranes but is later found in lysosomes and is finally degraded; therefore, it is conceivable that different phases in the disease progression might account for differences in staining patterns; (3) the disease process might be different between both species; and (4) finally, post-mortem alterations might be responsible for decreased signal intensity in human samples.

Cadherins are members of a family of adhesion molecules involved in calcium-dependent homotypic cell–cell interactions [4]. E-cadherin is a 120-kDa glycoprotein that is highly expressed during development and is the predominant cadherin of the liver epithelium. It is concentrated in the adherens junction, where it interacts with actin filaments. In the absence of E-cadherin, epithelial cells are not capable of a stable intercellular adhesion.

Our results suggest an irregular expression of E-cadherin in liver cells in human leptospirosis. This alteration might be primarily related to cell membrane lesion or secondary to other hepatocyte injuries. In either case, the persistence, at least partially, of E-cadherin expression might be a marker for restoration of a normal liver cell membrane function in cases of recovery from the disease and merits attention as a potential prognostic marker and/or factor.

Liver cell-plate disarray, when present at autopsy, is a major histopathologic marker of liver involvement in leptospirosis [5, 8, 24]. However, it was not observed in biopsy studies [1, 10, 11] and its absence raised the hypothesis that this aspect might appear only at a more acute phase of the disease or during the agonal period. The proponents of this idea argued that human biopsies could only be performed when the hemorrhagic phase had subsided and the patients, therefore, were in an early recovery phase of the disease. Furthermore, experimental work on a sequential daily basis validated the liver-plate disarray [3, 20]. Taken together, these results suggest that, even if agonal and post-mortem changes can worsen the liver pattern, the lesion is genuine and regresses rapidly, which justifies its absence in biopsy studies.

Deposits on the host cell membrane of LAg and loss of E-cadherin membrane expression in leptospirotic patients might corroborate previous suggestions that cell membrane damage is the primary lesion in leptospirosis, mediated by a glycolipoprotein (GLP) [12, 26, 27] or lipoproteins transported to the leptospiral surface and/or secreted [22]. In agreement with these suggestions is the demonstration that GLP in the experimental model has also an intense affinity for hepatocyte cell membranes and is detected by IHC only at the late phase of the disease as granules both in the cytoplasm of macrophages and on the cell membranes, suggesting that its presence is the result of partial leptospiral degradation secondary to phagocytosis [2, 3, 23]. Vinh et al. [27] suggested that such adherence of leptospires and/or their products might lead subsequently to the microorganism fatty acids to be intercalated into the cell membrane. It is worth remembering, however, that leptospires have, as the major carbon and energy sources, long-chain fatty acids from which energy is derived mainly by beta-oxidation [14]. Therefore, as an alternative to Vinh's [27] suggestion, we might speculate that the microorganism's adherence to cell is necessary to supply its energy requests which would be provided by cell membrane fatty acids.

ISH detected sequences of leptospires chiefly in the nuclei of liver cells in human cases. The relevance of this finding, if any, is unknown at the moment. However, when we take into account the occasional human and experimental ISH findings showing also a cytoplasmic signal together with the immunoelectron findings of the experimental disease [12], it is possible to suppose that leptospiral nucleotide sequences, initially localized on the cell membrane and subsequently internalized by the endocytic lysosomic pathway, might migrate to the nucleus. Supporting this hypothesis, Darji et al. [9], working with an attenuated strain of *Salmonella typhimurium*, observed a similar nuclear staining pattern and suggested that lysis of bacteria in the phagolysosome compartment and escape to the cytosol of the host cell should precede the plasmid DNA transfer into the nucleus. Moreover, Holmgren et al. [17] demonstrated that cultivation of apoptotic bodies derived from Epstein–Barr virus (EBV)-carrying cell lines with either fibroblasts, monocytes, or endothelial cells resulted in the uptake of DNA and expression of EBV-specific markers in the recipient cells. Therefore, it is conceivable that specific nuclear staining could be visualized in leptospirotic patients. The weaker signal observed with ISH when compared to IHC might have several explanations: (1) IHC reactions were performed using amplification-based protocols, which usually increase the signal by several folds and (2) the LAg sera were obtained from whole *Leptospira*, and it is expected to recognize several

different epitopes, which would result in stronger signal when compared to the single-sequence-based small ISH probes.

The characteristics mentioned above might render the IHC reactions prone to nonspecific results. Our results, however, confirm that the available antibodies are specific and might be used, as previously reported [16, 29], for diagnostic purposes in situations in which leptospirosis is suspected and/or to study disease process in leptospirosis animal models [1–3, 20]. ISH and IHC apparently can detect leptospirosis with the same sensibility. The unique ISH staining pattern, however, suggests that this technique might be more useful in human and experimental pathogenetic assays.

In conclusion, this study brings further support to the idea that cell membrane lesion might play an important role in leptospirosis pathogenesis. Furthermore, we show herein that the loss of E-cadherin membrane expression is associated with liver-plate disarray, stressing that this feature is a genuine lesion in leptospirosis and suggesting that this finding might have prognostic relevance. Finally, we suggest that IHC and ISH might be used for both diagnostic and research purposes.

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