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Review Article

Pathogenesis of Pulmonary Hemorrhagic Syndrome in Human Leptospirosis

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Abstract. Based on a previous study and by incorporating new knowledge, the goal of our study was to understand more fully the pathogenesis of hemorrhagic pneumonia of severe human leptospirosis, highlighting the onset of capillary lesions by *Leptospira* itself and/or its antigenic/toxic products acting on the endothelium and binding to cadherins. Both events lead to loss of endothelial integrity, alter permeability, cause rupture, and open intercellular junctions, contributing to the hemorrhagic phenomena associated with severe leptospirosis.

Leptospirosis is a worldwide zoonotic infection caused by pathogenic spirochetes of the genus *Leptospira*. Rodents play an important role in the epidemiology of the disease as chronic carriers, persistently harboring *Leptospira* in their proximal kidney tubules and excreting live bacteria into the environment, thus contaminating water and soil. When humans come into contact with contaminated water or soil, these highly motile bacteria can penetrate injured skin or mucous membranes and, after complement evasion,[1] disseminate through the hematogenic route causing systemic infection.

In Brazil, leptospirosis is endemic and becomes epidemic during rainy periods, mainly affecting low-income metropolitan areas of capital cities as a result of flooding, inadequate sanitation, and high infestation of infected rodents. According to the Brazilian Health Ministry, there were 3,368 confirmed cases and 280 deaths in 2019, and a total of 48,670 confirmed cases and 4,287 deaths from 2007 to 2019.[2]

Several pathogenic species of the genus *Leptospira* can cause a wide range of acute clinical manifestations, from mild flu-like illness to a severe form of disease called Weil's syndrome, characterized by jaundice, renal failure, decreased platelet count, bleeding, hypotension, and severe pulmonary hemorrhagic syndrome, which is a major cause of death. Pulmonary involvement in leptospirosis manifests as intra-alveolar hemorrhage and acute respiratory distress syndrome that sometimes requires intubation and mechanical ventilation. Strategies such as early renal replacement therapy and lung protective ventilation using a low tidal volume, with an initial tidal volume of 6 mL/kg, a positive end expiratory pressure of 5 cm H_2O, and a limited plateau pressure of $\leq$ 30 cm H_2O can improve outcomes.[3]

It is known that the initial process of leptospiral adhesion to biomolecules present on epithelial cells is an important step for host colonization and invasion. The interaction of *Leptospira* with several components of the extracellular matrix (ECM) and cell surface receptors has been investigated previously.[4–6] In a study by Nicodemo et al.,[7] lung fragments from 12 patients with leptospirosis were collected immediately after death, and were studied by light and electron microscopy, and by immunohistochemistry to describe the morphological and ultrastructural aspects of the lung and platelets in leptospirosis. The ultrastructural findings were uniform and constant. Capillary lesions were characterized by swelling of the endothelial cells, an increase in pinocytotic vesicles, giant dense bodies in the cytoplasm, and emission of pseudopods, showing clear signals of morphological activation of these cells in human leptospirosis (Figures 1 and 2). Leptospiral antigen was detected in eight cases as positive granular material on the luminal surface of the endothelium and in the cytoplasm of the endothelial cells of septal capillaries, and in the filamentous form, attached to the endothelium of the septal capillaries (Figures 3 and 4). Therefore, the pneumopathy of human leptospirosis seemed to be specific and triggered initially by *Leptospira* (Figure 5) and/or by their antigenic/toxic products interacting with endothelial cell surface receptors and/or the ECM. Nicodemo et al.[7] concluded that lung involvement in severe human disease presents as hemorrhagic pneumopathy with septal capillary injury (Figure 6), which should be considered the most important factor in the pathogenesis of the bleeding disturbances.

Researchers have been trying to understand more fully the mechanisms by which pathogenic *Leptospira* interact with and alter the endothelium. One such mechanism of adhesion to host cells is the capacity of attachment to the cadherins, which are a family of calcium-dependent transmembrane

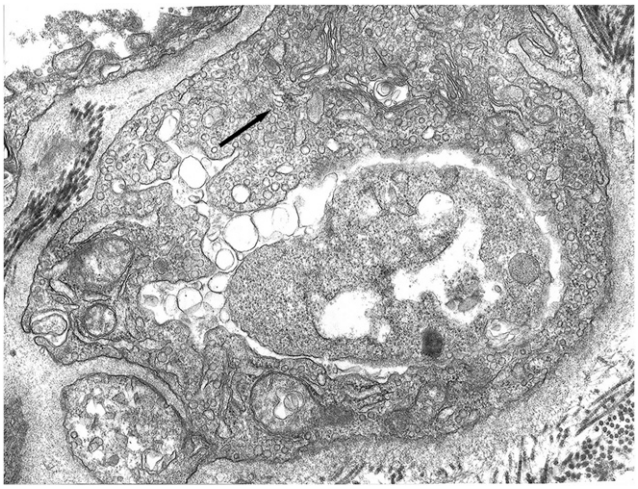

FIGURE 1. Septal capillary with edema and increased pinocytotic vesicles in endothelial cell, ×81.900 original magnification.

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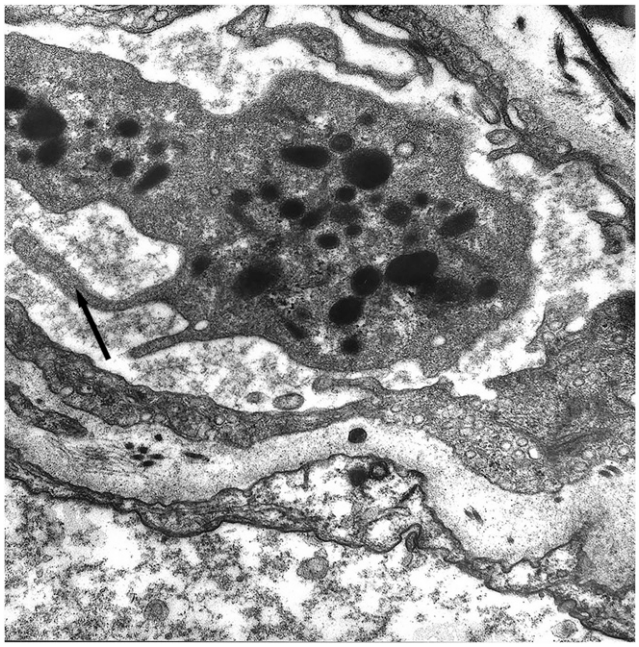

FIGURE 2. Septal capillary with pseudopod emission by the endothelial cell and platelet, ×42.560 original magnification.

adhesion proteins that function to maintain cell–cell integrity and serve as receptors for *Leptospira interrogans*. *Leptospira* bind to vascular endothelial cadherin located at the intercellular junctions and to neural cadherin distributed primarily across the cell surface. Both events can lead to a loss in endothelial integrity, altered permeability, endothelial disruption, opening of intercellular junctions, and vascular leakage, contributing to the pathogenesis of hemorrhagic syndrome.[8–11]

Multiple *Leptospira* adhesins have been reported to bind cells via cadherins or the ECM molecules fibronectin, collagen, laminin, elastin, and plasminogen.[12] According to Kochi et al.,[11] the recombinant proteins coded by the genes *LIC 11711* and *LIC 12587* of *L. interrogans* serovar Copenhageni

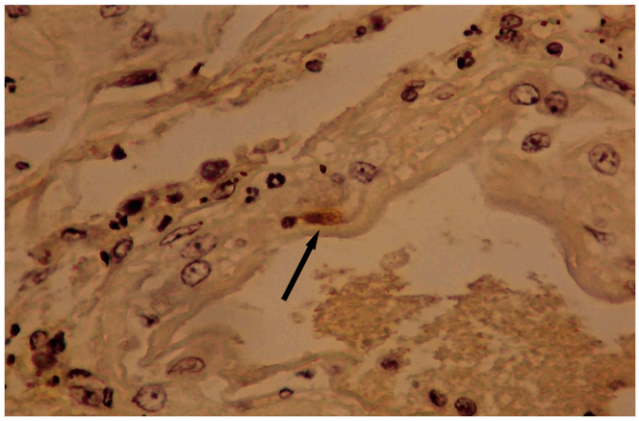

FIGURE 3. Granular leptospiral antigen (arrow) in an endothelial cell of pulmonary capillary; avidin–biotin stained, ×600 original magnification. This figure appears in color at www.ajtmh.org.

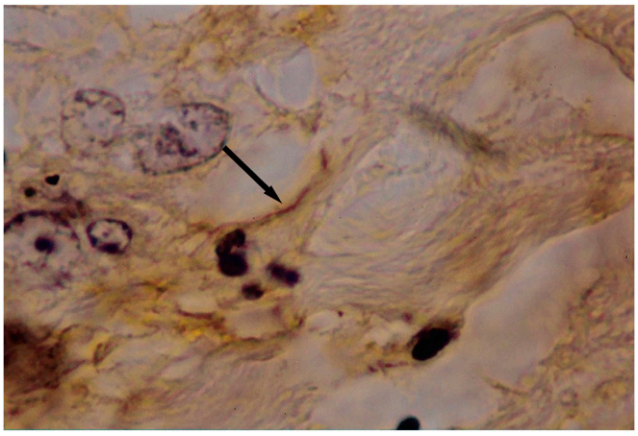

FIGURE 4. Filamentous leptospiral antigen (arrow) attached to the endothelium of a septal capillary; avidin–biotin stained, ×1,000 original magnification. This figure appears in color at www.ajtmh.org.

are conserved among pathogenic strains and are probably surface-exposed in the bacterial surface, and were characterized as novel E-cadherin ligands.

Bacterial attachment to ECM components is an important step for the possible role of this specificity for ECM molecules; some are exclusive laminin-binding proteins whereas others have broader spectrum-binding profiles (Lig B, Lsa 21, Lip L53). These proteins also may play a role in the colonization of host tissues.[3–12]

From published research, severe pneumopathy of human leptospirosis appears to be specific and triggered directly by *Leptospira* and/or their antigenic/toxic products acting on endothelial cell surface receptors or even interacting with components of the ECM. Deposits of leptospiral antigen on host cell membranes and alteration of cadherin expression lead to loss of vascular integrity, vascular leakage, invasion, dissemination, and involvement of multiple organs and tissues, causing severe illness.

Further studies are needed to clarify the importance of the various proteins expressed by pathogenic *Leptospira* during infection and their relation to virulence and pathogenicity to

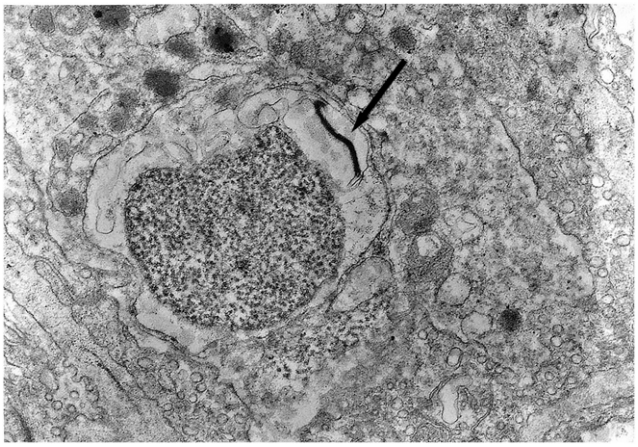

FIGURE 5. Leptospira (arrow) next to the mononuclear cell in septal capillary; conventional electron microscopy, 2.1 × 19.500.

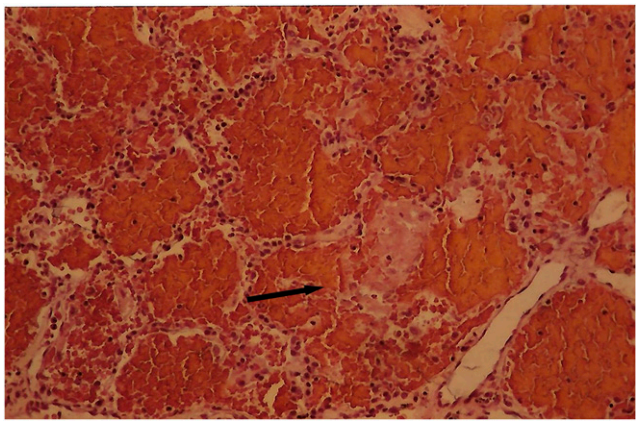

FIGURE 6. Alveoli filled with erythrocytes and ruptured alveolar septa; hematoxylin eosin stain, ×150 magnification. This figure appears in color at www.ajtmh.org.

improve our understanding of the pathogenesis of human leptospirosis.

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