Review

Leptospirosis-associated disturbances of blood vessels, lungs and hemostasis

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Abstract

The frequency of massive pulmonary hemorrhages seems to be increasing in different geographic areas; however, there is no clear explanation for this trend. Although data on the pathogenesis of such complications are scarce, recent research indicates a potential role of autoimmunity and/or multifactorial mechanisms. However, much information is already available on the disturbance of hemostasis and blood vessels in leptospirosis-related literature, even if some contradictory concepts coexist. The purpose of this review is to integrate both new and classical information from human and animal studies on severe pulmonary forms of leptospirosis and disorders of hemostasis and blood vessels. We propose that the involvement of blood vessels in leptospirosis must be understood as a sepsis-like, diffuse process of endothelial activation/damage rather than as a classical systemic vasculitis. Pulmonary hemorrhages are most likely multifactorial and there has recently been evidence against the role of autoimmunity; however, further investigation of strain variations, exposure to hydrocarbons and association with renal dysfunction is required. Thrombocytopenia is a consistent feature of leptospirosis but it is not clear whether it is attributable to sepsis-related mechanisms. In addition, further investigation is required to define whether platelet function is activated or inhibited during severe leptospirosis.

Keywords: Leptospirosis, Disease, Pulmonary, Vasculitis, Hemorrhage

1. Introduction

Leptospirosis is a widespread zoonosis with global distribution that is caused by pathogenic spirochetes species of the genus Leptospira. The infection may be transmitted to humans by direct contact with infected tissues or contaminated urine, or indirectly by contact with water or moist soil contaminated with the urine of mammalian reservoirs. The disease occurs as rural endemics and flood-associated urban outbreaks, and it is associated with occupational and recreational exposure. However, leptospirosis is a zoonosis of worldwide importance and there are case reports from all over the world. Severe manifestations occur in 5–10% of human infections and present mainly as: (1) Weil’s syndrome, a triad of jaundice, hemorrhagic diathesis and acute renal failure (with 10–15% case fatality in most series); and (2) severe pulmonary hemorrhage syndrome (SPHS), which may present as acute respiratory distress syndrome (ARDS) or massive pulmonary hem-
orralge, with case fatality >50% in many series (McBride et al., 2005).

Although bleeding events are a common feature of leptospirosis, lung involvement, especially pulmonary hemorrhage, has been increasingly recognized worldwide after an outbreak of leptospirosis in Nicaragua with high rate of severe pulmonary hemorrhage in 1995 (Trevejo et al., 1998). There is no clear explanation for this trend. Hemorrhages in general, and pulmonary hemorrhage in particular, have a major impact on disease prognosis; however, their pathogeneses remain unclear. Although recent data have expanded our understanding of the potential mechanisms of hemostatic disorders in leptospirosis, a body of contradictory information is available.

The purpose of this review is to integrate both classical and new information from human and animal studies to discuss the available data on severe pulmonary forms of leptospirosis and disorders of hemostasis and blood vessels.

2. Blood vessels and systemic inflammation

Leptospirosis is commonly assumed to cause systemic vasculitis, which would be one of the main mechanisms of tissue damage. Inflammation of the vascular walls may be a consequence of the direct invasion of infectious agents, or of immune mechanisms such as immune complex deposition, auto-antibodies and cell-mediated immunity. None of these pathways have been clearly documented in the vascular lesions seen in leptospirosis.

An experimental attempt to study the ultrastructure of blood vessels in leptospirosis reported that the presence of endothelial lesions was an early feature that lead to disturbed permeability (De Brito et al., 1979). Diffuse alveolar damage, the morphological counterpart of ARDS, is a frequent feature of leptospirosis (Arean, 1962a; Nicodemo et al., 1997; Salkade et al., 2005). The concept that acute leptospirosis can cause diffuse activating endothelial disorder places it closer to the pathogenesis of sepsis than to systemic vasculitis.

A systemic inflammatory response is a probable feature of leptospirosis. In a series of 18 patients from São Paulo, Brazil, the serum levels of tumor necrosis factor α (TNF-α) were related to mortality. In this report, only four patients had detectable serum levels of the cytokine by ELISA on the first or second day of hospitalization, and three of them had a fatal outcome. Only patients with detectable levels of TNF-α had clinical manifestations of kidney, liver and lung involvement or hemorrhages (Tajiki and Salomao, 1996). Patients with severe leptospirosis also have high serum levels of nitric oxide, an important mediator of endothelial response in sepsis, and these high levels correlate with an elevation of serum creatinine (Maciel et al., 2006). More recently, in a series of 52 patients from Indonesia, the inflammatory markers long pentraxin PTX3, IL-6 and IL-8 were associated with a lethal outcome and PTX3 was also associated with disease severity (Wagenaar et al., 2009). These data suggest the existence of a systemic inflammatory response in human leptospirosis that results in a pathology similar to that seen in meningococcemia (Hotchkiss and Karl, 2003). In severely ill patients, the most common hemodynamic disorder is the typical sepsis pattern of a combined high cardiac index and low systemic vascular resistance (Siriwanij et al., 2005). In the above-mentioned study from Indonesia, 24 (46%) of the patients fulfilled the criteria for sepsis, 17 (3%) for severe sepsis and 7 (14%) for septic shock (Wagenaar et al., 2009).

Besides diffuse endothelial damage and systemic inflammation, reports of true attacks on arterial walls are also available. Marked inflammation in aortic adventitia was noted in 4/33 (12%) and 11/19 (58%), and coronary inflammation in 2/33 (6%) and 14/20 (70%) patients, in human necropsy series (Arean, 1962a; de Brito et al., 1987). There are some descriptions of the pathologic anatomy of cerebral arteritis in leptospirosis from China (Chen, 1990; Gong, 1984). The inflammation in this disease may involve all layers of the main branches of large arteries in the base of the brain, and it was found to occur in both children and adolescents, leading to a multiple occlusive cerebrovascular disease in 9/12 (75%) cases in the largest series of so-called cerebrovascular leptospirosis (Chen, 1990).

In an experimental guinea pig model, Wakamatsu et al. described myolysis and leukocytic infiltration in the walls of renal arteries/arterioles and hepatic portal tracts (Wakamatsu et al., 1990). In TLR4-deficient C3H/HeJ mice, pulmonary hemorrhage paralleled vasculitis in the lung tissue and fibrinoid deposits in the wall of the small lung arteries were described, while larger vessels exhibited segmental inflammation (Pereira et al., 1998). Such findings have not been reported in humans and, further, the role of TLR4 in the protection against leptospiroinfection and the pathogenesis of leptospirosis is still poorly understood, which complicates the interpretation of these observations.

The above reports are relevant as they suggest that vasculitis may be an important feature in some cases of leptospirosis. However, most studies in human and experimental leptospirosis infections do not support vasculitis as a constant primary event that causes tissue damage. Indeed, most of the lesions seen in leptospirosis would probably be better explained as a direct effect against parenchymal cells or as direct endothelial damage with increased vascular permeability.

3. Severe pulmonary forms

3.1. An emerging, severe clinical form?

The microscopic finding of intra-alveolar hemorrhage is frequent in both human and experimental leptospirosis (Arean, 1962b; Nally et al., 2004; Pereira et al., 2005), ranging from the clinically undetectable to a dramatic presentation of massive hemoptysis and respiratory failure with rapid evolution. The presence of severe pulmonary hemorrhage in leptospirosis is not new; it has long been known to be a common manifestation of leptospirosis in China and Korea (Faine et al., 1999; Park et al., 1989), but the body of literature suggests that it is an emerging form of this disease in other countries, such as Nicaragua and Brazil (Gouveia et al., 2008; Trevejo et al., 1998). The severe hemorrhagic lung form of leptospirosis may occur in outbreaks or sporadic cases, and may or may not be coincident with icteric disease (Marotto et al., 1999; Segura et al., 2005; Silva et al., 2002; Trevejo et al., 1998). The pathogenesis of pulmonary hemorrhage due to leptospirosis is still being investigated.

The presentation of leptospirosis seems to be distinct in different geographical areas worldwide. The incidence of pulmonary hemorrhage is probably underestimated and could be better estimated by necropsy-based studies. In rural epidemics in Nicaragua and endemic disease in Peru, SPHS presents without the classic accompanying manifestations such as jaundice and renal failure (Segura et al., 2005; Trevejo et al., 1998). In Iquitos, Peru, seven patients were detected with pulmonary hemorrhage, which was probably the main cause of death, and the failure of physicians to recognize leptospirosis has been highlighted and attributed to the lack of typical symptoms (Segura et al., 2005). In the city of Salvador, Brazil, SPHS has emerged as the major cause of death in recent years (55% in 2005) (Gouveia et al., 2008). Interestingly, cases of pulmonary hemorrhage were not detected in Salvador until 2003. Among the 143 lethal cases of leptospirosis in the infectious disease referral hospital of Salvador from 1993 to 1997, 76% of deaths were attributed to renal failure while gastrointestinal bleed-
ing, the second most common cause, accounted for 10% (Costa et al., 2001). This observation is relevant because it shows that pulmonary hemorrhage cannot be solely explained by the underlying leptospirosis-related coagulopathy. An active surveillance has been employed in Salvador for 13 years and the emergence of SPHS is unlikely to be explained by underdiagnosis. In the city of São Paulo, Brazil, where necropsy is performed on most lethal cases, necropsied cases usually (72%) share features of Weil's syndrome and SPHS, and pulmonary hemorrhage has long been recognized as a common, severe feature of leptospirosis (Spichler et al., 2007).

These observations of a true emergence of SPHS may be explained by the introduction of new strains in a determinate geographic area. A molecular epidemiology study from India observed that clinical isolates with a different disease presentation (mild symptoms or severe manifestations including pulmonary hemorrhage) could be distinguished by using random amplified polymorphic DNA (RAPD) fingerprinting (Natarajaseenivasan et al., 2005). The hypothesis that specific strain variants may explain different clinical forms of leptospirosis must be tested in other settings. To date, there is no convincing evidence that different serovars have the intrinsic tendency to cause distinct presentations of leptospirosis (Vinetz, 2001). In Brazil, serovar Copenhageni predominates as the major cause of severe urban leptospirosis, sharing a similar genetic profile in different urban areas (Pereira et al., 2000), and the frequency of severe pulmonary forms is quite variable between the largest Brazilian cities. In addition, strains of serovar Copenhageni isolated from patients with and without SPHS can cause variable rates of pulmonary hemorrhage under experimental conditions in laboratory animals (Nally et al., 2004; Silva et al., 2008).

In a 3-year (2004–2006) population-based study on severe cases of leptospirosis in São Paulo, Brazil, severe pulmonary forms were observed in 74% of lethal cases and 26% of survivors, making it the strongest predictor of lethal outcome (OR: 9.1) (Spichler et al., 2008c), which is agreement with other reports from places where severe pulmonary forms are also common (Paganin et al., 2007). Some interesting clinical associations were suggested from this experience. The strongest risk factor for pulmonary involvement was oliguria. The mean platelet count was significantly lower in patients with pulmonary involvement, and those patients more frequently had platelet counts <70,000 units per mm$^3$, jaundice and serum creatinine levels >3 mg/dl. This finding suggests that oliguric ARF is, at the very least, an additional factor that may exacerbate/accelerate specific alveolar damage induced by either the direct toxic effects of leptospires or an underlying autoimmune process (Spichler et al., 2008a). Reports from a large case studies from São Paulo indicates a role of uremia in lung involvement, as was previously suggested by other researchers in reports that were hampered by either small sample sizes or poor study design (Chen et al., 2007; de Carvalho et al., 1992; Niwattayakul et al., 2002; Pereira da Silva et al., 1976). When comparing all survivors with those lethal cases in which pulmonary hemorrhage was interpreted as the cause of death at necropsy (n = 31), the following difference was significant: lethal pulmonary hemorrhage was associated with lower platelet counts and more frequently had a count of <70,000 units of platelets per mm$^3$ at admission (69% vs. 37%) (Spichler et al., 2008a). The previous observation of a relationship between thrombocytopenia and ARDS was reported in a case series from Thailand (Thammakumpee et al., 2005). Taken together, these findings indicate a multifactorial mechanism for lung involvement in leptospirosis, with uremia and thrombocytopenia also potentially playing a role.

Little information is available on lung involvement in children. In São Paulo, The coexistence of oliguria and respiratory involvement was observed in 9/42 pediatric patients aged <18 years, accounting for 23% of the cases in this age group from 2003 to 2006. Only one case was <12 years of age. Case fatality in this condition was 1/9 (11%). The same presentation of oliguria with respiratory involvement in adults had a fatality rate of 55/93 (59%) (Spichler et al., 2008b). Thus, the case fatality of the combined renal and pulmonary forms is lower in this age group despite the fact that this severe presentation may be observed in nearly 1/4 of severe pediatric cases.

### 3.2. Pathology and pathogenesis of severe pulmonary forms

An ultrastructural investigation in 12 human necropsies described endothelial damage in lung tissue as a constant and uniform feature (Nicodemo et al., 1997). ARDS has been described in some clinical series (Bharti et al., 2003; McBride et al., 2005). A recent report of 40 necropsies of confirmed leptospirosis cases from India described gross pulmonary hemorrhage in 34/40 (85%) cases, while 4/40 (10%) had a marked presentation of hyaline membranes—a typical feature of ARDS (Salkade et al., 2005). In a previous study described by Arean, 5/33 cases (15%) presented hyaline membranes (Arean, 1962a). In 12 necropsies from São Paulo, Brazil, immunohistochemistry detected fibrin deposits in the alveolar walls of seven cases, providing further evidence of diffuse alveolar damage (Nicodemo et al., 1997). In a recent series from São Paulo that described 30 necropsies of leptospirosis-associated pulmonary hemorrhage cases, fibrin deposition was detected in 28/30 cases and one observed hyaline membranes was detected in all cases (Croda et al., 2009). A recent experimental attempt to reproduce alveolar damage in the hamster model showed that the progression of lung lesions paralleled the increasing expression of TNF and endothelial nitric oxide synthase (Marinho et al., 2009). Taken together, these data indicate a role of local and/or systemic inflammation in the pathogenesis of severe pulmonary forms of leptospirosis.

Nally et al. explored an immunologically mediated mechanism of pulmonary hemorrhage in leptospirosis using the guinea pig model. Animals experimentally infected by *Leptospira interroga*-tans serovar Copenhageni exhibited lung hemorrhages consistent with those seen in human disease and had linear deposits of immunoglobulin and complement along the septal matrix. Of a total of 22 infected animals, 19 had at least one class of immunoglobulin deposited along the alveolar septa. The linear deposits of complement (C3) were invariably related to at least two classes of antibodies, suggesting that its presence was secondary to antibody binding to target antigens in *situ* (Nally et al., 2004). This pattern is similar to what is described for Goodpasture’s syndrome, in which cross-reacting antibodies against renal glomerular basal membrane (GBM) and lung septal matrix cause massive hemoptysis. While the lung pathology of experimental leptospirosis is quite similar and both diseases share similar respiratory manifestations, there are no significant glomerular lesions seen in either human or experimental leptospirosis.

The relevance of anti-GBM antibodies in clinical situations other than Goodpasture’s syndrome seems to be controversial. Recent data suggest that anti-GBM antibodies may be found in healthy humans (Cui et al., 2006; Yang et al., 2007). Following the study on guinea pigs, a single human necropsy from Taiwan was reported in which linear deposits of IgG and IgM along the alveolar septa were detected (Yang and Hsu, 2005). Unfortunately, this study lacked the appropriate negative controls.

In a recent necropsy series from São Paulo, Brazil, 30 lung samples from lethal cases of leptospirosis-associated pulmonary hemorrhage were compared to seven lung samples from patients with lethal pulmonary hemorrhage of other etiologies. It was reported that the lungs of patients with leptospirosis exhibited more alveolar macrophages, hyaline membranes, necrosis of alveolar surfaces, type II pneumocyte regeneration, and plasma cells...
at the alveolar septa. The deposition of antibody and complement was detected in 17/30 patients: 12 with a linear and 5 with a focal pattern of distribution along the alveolar surface. All 17 cases were positive for C3 and two classes of immunoglobulin (IgA, IgM or IgG). Importantly, the same study included a serum analysis of patients from Salvador and no difference was observed in the frequency of anti-GBM antibodies between controls and patients with leptospirosis, with or without SPHS. In addition, anti-GBM levels did not differ between the first and second sample collections, which were usually separated by 15 days during acute disease (Croda et al., 2009). In contrast, anti-cardiolipin IgG antibodies increased from the first to the second sample in the SPHS group (discussed below). In a more recent report, 40 patients with leptospirosis had negative serum tests for anti-GBM antibodies (Craig et al., 2009).

As it is possible that, in these instances, antibody deposition may have reflected autoimmunity or an epiphenomenon, it is important that appropriate human/animal controls are used. Because anti-GBM antibodies may occur in natural sera, any condition causing alveolar necrosis may favor their deposition. The best control group for the previous studies would be patients/animals with diffuse alveolar damage of other etiologies. In experimental models, the use of uninfected controls seriously inhibits the ability to form definitive conclusions (Nally et al., 2004). In the necropsy series from São Paulo, it is important to note that, even if more appropriate controls were used (patients with pulmonary hemorrhage of other etiologies), patients with leptospirosis still had a higher frequency of pneumocyte necrosis and hyaline membranes (Croda et al., 2009).

The potential role of an anti-GBM antibody mechanism in leptospirosis-associated lung disease opens some tempting hypothesis. For instance, exposure to hydrocarbons seems to result in altered forms of collagen IV that result in the presentation of new epitopes and subsequent autoimmunity against GBM (Jennette and Nickeleit, 2007). In a series of 26 patients from Spain, pulmonary involvement in leptospirosis was associated with cigarette smoking (Martinez Garcia et al., 2000); however, further clinical studies associating severe pulmonary forms of leptospirosis with cigarette smoking or other forms of solvent/hydrocarbon exposure are lacking.

The hypothesis that SPHS may be related to immunopathogenesis, specifically auto-antibodies, is challenged by the recent description of lethal disease with pulmonary hemorrhage in experimentally infected severe combined immunodeficiency (SCID) mice (Viriyakosol et al., 2006), which are unable to differentiate B and T lymphocyte subsets (Nally et al., 2005). Rats are the prototype of resistance to acute lethal infection (Athanazio et al., 2001), but, in agreement with the observation in these SCID mice, rats treated with cyclophosphamide (which invokes suppressed humoral immunity) develop pulmonary hemorrhage (Thiermann, 1980).

The emergence of SPHS in some areas could be alternately explained by the introduction of a new respiratory virus that predisposes patients both to the direct effects of leptospires in lung parenchyma and to the putative immunologically mediated lesions. Such a predisposition to these lesions could mirror the known relationship between seasonal viral infections and the incidence of some autoimmune/rheumatic diseases (Schlesinger et al., 2005; van der Werf et al., 2007). It has been suggested that previous exposure to hantavirus infection could be a triggering mechanism of severe pulmonary symptoms in leptospirosis. Such an association, however, has never been investigated except for a brief report that serologic evidence of a previous hantavirus infection was observed in patients with other infectious diseases in Salvador, Brazil (Costa et al., 2001).

Taken together, these data suggest that severe pulmonary forms and pulmonary hemorrhages are probably multifactorial, and that many of these factors have not been well-explored. Observations in guinea pigs have suggested a potential role of autoimmunity in pulmonary hemorrhages, but more recent studies in different animal models as well as patients have not supported an immune-mediated mechanism. In addition, it seems that this complication in humans occurs too early in the course of disease to be attributable to immunopathogenesis. Temporal and geographical variations in the rate of the severe pulmonary form may favor the hypothesis of an as yet unknown interaction with a respiratory virus or with leptospiral strain variations: both are currently unexplored fields in leptospirosis. The postulated associations with severe renal dysfunction and cigarette smoking and/or other forms of hydrocarbon exposure should be evaluated in future large clinical series.

4. Hemostatic system

4.1. A platelet-activating disorder?

As reviewed above, severe leptospirosis shares some clinical features, such as meningococcemia, with Gram-negative sepsis. Hemorrhages in these circumstances are usually related to a prothrombotic state that leads to disseminated intravascular coagulation (DIC). However, most human and experimental data do not support a consumption coagulopathy as the underlying mechanism of bleeding (Nally et al., 2004; Nicodemo et al., 1990; Yang et al., 2006).

In addition, it is well known that platelets are decreased in septic patients because of mechanisms unrelated to DIC. The degree of thrombocytopenia has been shown to correlate with both case severity and mortality (Ma and Kubes, 2008). Interestingly, we observed that this same thrombocytopenia was a strong predictor of lethal outcome in patients with severe leptospirosis from São Paulo (Spichler et al., 2008c). In a recent work, the mechanism postulated to explain the sepsis-associated thrombocytopenia was platelet–neutrophil cooperativity that led to the migration of both platelets and neutrophils to the lung and liver. The activation of the TLR4 signaling pathway in platelets stimulates their binding to neutrophils, leading to further neutrophil activation and the release of web-like structures named neutrophil extracellular traps (NETs) (Clark et al., 2007). The neutrophil–platelet interaction and the formation of NETs are important for trapping and killing pathogens, however, they also offer an alternative explanation for platelet depletion in severe bacterial infections (Ma and Kubes, 2008).

A potential role of endothelial damage and platelet activation in the hemostatic disorders seen in leptospirosis is highlighted by necropsy data from 12 patients with hemorrhagic manifestations. The electron microscopy study described pulmonary endothelial cell swelling, damage of lung microcirculation and morphological platelet changes that were suggestive of activation (Nicodemo et al., 1997). Endothelial damage/activation as the triggering mechanism of platelet activation and aggregation, without the disturbance of the coagulation cascade, would place the bleeding disorders of leptospirosis close to thrombotic thrombocytopenic purpura (TTP); however, the typical clinical presentation of TTP is rarely reported (Homs et al., 2003; Laing et al., 1990). One case report documented granular hyaline thrombi composed of platelets with no fibrin in small vessels of the brain, heart, lungs and kidneys at necropsy (Laing et al., 1990).

In a recent report using the guinea pig model, no fibrin or platelet thrombi were detected in the lung microcirculation; however, electron microscopy of the hepatic sinusoids detected platelet aggregation, deformed erythrocytes, and phagocytosis of both platelets and erythrocytes by Kupffer cells (Yang et al., 2006). The laboratory findings were suggestive of platelet activation (with elevated serum levels of 11-dehydrogenate thromboxane
B2, 11-DH-TXB2) and endothelial damage (elevated thrombomodulin), while the activation of thrombin (decreased levels of antithrombin–thrombin III complex) and fibrinolysis (D-dimer and fibrin degradation products slightly increased) ruled out disseminated intravascular coagulation (DIC). Nally et al. reproduced severe SPHS in guinea pigs and also observed hepatic necrosis, while inconsistent thrombocytopenia, an absence of high serum levels of fibrinolysis products, and serum levels of fibrinogen within the normal range ruled out the possibility of a consumption coagulopathy (Nally et al., 2004).

Silva et al. reported that, in guinea pigs, laboratory data did not correlate with clinically detectable hemorrhages and only microscopic foci were observed (da Silva et al., 1995). The authors suggested that extensive endothelial damage could be a possible trigger of hemostatic disturbances, based on the observation of extensive foci of liver coagulation necrosis associated with fibrin deposits. In agreement with this observation, hepatic cell necrosis was found to parallel bleeding events in a non-human primate model (Pereira et al., 2005).

There are, however, clinical reports that characterize DIC in leptospirosis. In a series of 13 necropsies from Rio de Janeiro, Brazil, fibrin thrombi were detected in several organs in 8 cases, including 6 cases in whom they could be demonstrated in lung tissue (tospirosis. In a series of 13 necropsies from Rio de Janeiro, Brazil, microscopic foci were observed (da Silva et al., 1995). The authors reported that, in guinea pigs, laboratory data did not correlate with clinically detectable hemorrhages and only microscopic foci were observed (da Silva et al., 1995). The authors suggested that extensive endothelial damage could be a possible trigger of hemostatic disturbances, based on the observation of extensive foci of liver coagulation necrosis associated with fibrin deposits. In agreement with this observation, hepatic cell necrosis was found to parallel bleeding events in a non-human primate model (Pereira et al., 2005).

More recently, as commented above, a serum analysis in experimental leptospirosis (Nally et al., 2004; Nicodemo et al., 1990). Immune-mediated mechanisms have been poorly explored, although anti-platelet antibodies have been reported in patients with leptospirosis (Davenport et al., 1989). In a series of 30 consecutive patients from São Paulo, 3/30 (10%) patients with severe disease had circulating anti-platelet antibodies (Nicodemo et al., 1990). In a series of 30 patients from Fortaleza, Brazil, 86% of all subjects had hemorrhages, 64% had anti-cardiolipin IgM and 78.5% had detectable IgG (De Francesco Daher et al., 2002). A serologic study in Salvador, Brazil, reported that 9/39 (23%) patients with leptospirosis had elevated levels of anti-cardiolipin antibodies while 6/34 (17%) had anti-β2 glycoprotein I (anti-β2GPI) antibodies. In the same evaluation, 16/30 (53%) of patients with visceral leishmaniasis presented high levels of anti-β2GPI antibodies. Thrombotic events were observed only among systemic erythematous lupus patients with detectable anti-β2GPI antibodies; they were observed in no patients with detectable serum autoantibodies who had leptospirosis, syphilis or visceral leishmaniasis (Santiago et al., 2004).

Antiphospholipid antibodies are commonly associated with clinical thrombosis. It should be noted that there is no data to support the relationship between thrombosis and bleeding events in leptospirosis. Clinical reports of symptomatic thrombotic complications of leptospirosis are rare (Turhan et al., 2006).

More recently, as commented above, a serum analysis in patients from Salvador revealed that the level of anti-cardiolipin IgG antibodies raised during acute disease in patients with SPHS (Croda et al., 2009). Anti-cardiolipin antibodies are implicated in severe pulmonary disease, including bland pulmonary hemorrhage and pulmonary hemorrhage associated with capillaritis (Green et al., 1996; Stojanovich, 2006; Zamora et al., 1997). However, anti-cardiolipin antibodies are known to be nonspecific markers of different infectious diseases. Furthermore, recent data does not indicate that there is a major role of antiphospholipid antibodies in vascular pathology, suggesting that they may reflect epiphenomena in diverse conditions (Ankri et al., 1999; Endler et al., 2006).

4.2. A platelet inhibitory disorder?

Based on the observation that the leptospiral genome harbors genes that encode proteins sharing similarities with mammalian hemostatic factors, the first experimental attempt to test the role of one of these genes on hemostatic disturbances in leptospirosis focused on leptospirosis pathogenesis. PAF is a potent mediator of platelet aggregation, inflammation and anaphylaxis, while it is naturally inactivated by a group of acetylhydrolases. The characterization of these genes in serovar Lai reported that: (1) the gene does exhibit a PAF acetylhydrolase activity in vitro models, with kinetic parameters that are comparable to its human counterpart; (2) the gene is not located outside of the cell, implying that it is only released after bacterial lysis; and (3) the gene is present in diverse serovars, regardless of their pathogenic or saprophytic behavior, and their products share similar PAF-AH activity. The role of leptospirosis PAF-AH during infection, however, is not clear from the first studies on experimental infection in gerbils or preliminary data from patients. PAF-AH levels indeed increase after the experimental infection of gerbils, but in humans this increase only occurs in patients infected by icterohaemorrhagiae serogroup. Most PAF-AH detected in serum was not derived from leptospires. The authors suggested that in “small niches” the released lep-
endothelial damage is an early feature of experimental leptospirosis (De Brito et al., 1979). Diffuse alveolar damage is a common complication of leptospirosis (Arean, 1962a; Nicodemo et al., 1997; Salkade et al., 2005). Serum markers of systemic inflammations are related to severity of human leptospirosis (Tajiki and Salomao, 1996; Maciel et al., 2000; Wagenaar et al., 2009).

### Hemodynamic pattern of severe cases

Hemodynamic pattern of severe cases combines high cardiac index and low systemic vascular resistance (Siriwanj et al., 2005).

### Vascular disorders in leptospirosis resemble sepsis

**Pro**

- Endothelial damage is an early feature of experimental leptospirosis (De Brito et al., 1979).
- Diffuse alveolar damage is a common complication of leptospirosis (Arean, 1962a; Nicodemo et al., 1997; Salkade et al., 2005).

**Cons**

- None

### Vasculitis is an important cause of leptospirosis-associated lesions

**Pro**

- Aortitis and coronary vasculitis may be detected in 6–70% of human necropsies (Arean, 1962a; de Brito et al., 1987).
- There are rare case reports of cerebral arteritis (Chen, 1990; Gong, 1984).

**Cons**

- TLR4-deficient CH/HeJ mice develop small and large vessel vasculitis in the lungs (Pereira et al., 1998).
- Hamsters develops inflammation in the renal arteries/arterioles and hepatic portal tracts (Wakamatsu et al., 1990).

### Pulmonary hemorrhage is related to autoimmunity

**Pro**

- In guinea pigs, linear deposits of antibody and complement along alveolar septae resemble the autoimmune Goodpasture’s syndrome (Nally et al., 2004).
- Anti-cardiolipin IgG antibodies increase in patients who develop pulmonary hemorrhages (Crola et al., 2009).

**Cons**

- Linear deposits of antibody and complement in alveolar septae is not a constant feature in human leptospirosis (Crola et al., 2009).
- Anti-glomerular basal membrane antibodies are not elevated in patients with severe pulmonary involvement (Croda et al., 2009; Craig et al., 2009).

### Pulmonary hemorrhage is related to exposure to hydrocarbons

**Pro**

- Pulmonary involvement in leptospirosis was associated with cigarette smoking in Spanish patients (Martinez Garcia et al., 2000).

**Cons**

- Such observation was not observed or evaluated in other clinical studies.

### Pulmonary hemorrhage is related to uremia

**Pro**

- In São Paulo, the strongest risk factor for leptospirosis-associated pulmonary involvement is oliguria (Spichler et al., 2008a).
- Pulmonary involvement has been associated with hypotension and renal failure (Chen et al., 2007; Niwattayakul et al., 2002).

**Cons**

- Pulmonary hemorrhage in leptospirosis may occur unassociated with jaundice and renal failure in both outbreak or endemic settings (Treviño et al., 1998; Segura et al., 2005).

### Leptospirosis is a platelet-activating disorder

**Pro**

- Leptospirosis in guinea pigs is associated with elevated serum marker of platelet activation: 11-DH-TXB2 (Yang et al., 2006).
- Patients exhibit an increased thrombin–antithrombin complexes/plasmin–antiplasmin complexes ratio (Wagenaar et al., 2010).

**Cons**

- Patients exhibit platelets with morphological features of activation (Nicodemo et al., 1997).
- Uremia is a well-known cause of platelet dysfunction. Uremia has been associated with leptospirosis-associated thrombocytopenia (Edwards et al., 1982; Nicodemo et al., 1990; Spichler et al., 2008a) and may predict hemorrhages better than platelet depletion (Nicodemo et al., 1990).

### The leptospiral genome encodes proteins that can interfere with host hemostasis

**Pro**

- The genome encodes orthologs of platelet activating factor acetylhydrolase (PAF-AH), domain A of von Willebrand Factor, paraoxonase and a collagenase (Ren et al., 2003).
- Leptospiral PAF-AH cleaves the human platelet activating factor in vitro (Yang et al., 2009).

**Cons**

- Serum PAF-AH increases during experimental leptospirosis, however, it is not associated with hemorrhages and only a small fraction of the detected PAF-AH derives from leptospires (Yang et al., 2009).

### Hemorrhages are related to disseminated intravascular coagulation

**Pro**

- Diffuse alveolar damage is a common complication of leptospirosis (Arean, 1962a; de Brito et al., 1987).
- Leptospirosis in guinea pigs is associated with elevated serum marker of platelet activation: 11-DH-TXB2 (Yang et al., 2006).

**Cons**

- Patients exhibit platelets with morphological features of activation (Nicodemo et al., 1997).
- Uremia is a well-known cause of platelet dysfunction. Uremia has been associated with leptospirosis-associated thrombocytopenia (Edwards et al., 1982; Nicodemo et al., 1990; Spichler et al., 2008a) and may predict hemorrhages better than platelet depletion (Nicodemo et al., 1990).

### Proinflammatory response in leptospirosis

- Older studies from Brazil and Barbados are not supportive of disseminated intravascular coagulation in leptospirosis (Edwards et al., 1982; Nicodemo et al., 1990; Spichler et al., 2008a) and may predict hemorrhages better than platelet depletion (Nicodemo et al., 1990).

### Platelet depletion in leptospirosis

- Low platelet counts were associated with renal failure in a series of 36 cases from Barbados (Edwards et al., 1982).
- In São Paulo, lower levels of platelets were associated with elevated levels of blood urea and the requirement of dialysis (Nicodemo et al., 1990).
Strikingly, uremia, but not thrombocytopenia, could predict bleeding events in this case series. In São Paulo, oliguria and blood creatinine >3.0 mg/dl were independent risk factors for the development of thrombocytopenia (Spichler et al., 2008a). Alternatively, platelet dysfunction, rather than platelet depletion, may be the major hemostatic disorder in uremia (Hassan and Kroll, 2005).

Thus, it remains to be elucidated whether hemostatic disorder and thrombocytopenia in leptospirosis share the same mechanisms of sepsis or represent a unique form of coagulopathy determined by the leptospiral genome. A large body of evidence excludes the idea that the consistent thrombocytopenia seen in leptospirosis is related to DIC, but new clinical studies have suggested that at least a fraction of patients develop activation of the coagulation pathway and fibrinolysis. A sepsis-like entrapment of neutrophils and platelets in lung and liver circulation is also a potential explanation that is unexplored in leptospirosis. The genome of *L. interrogans* harbors some genes that are postulated to be involved in the disruption of host hemostasis; however, the first attempt to test this hypothesis showed little evidence that the leptospiral-derived PAF-AH are involved in the disease. In addition, it is not yet clear if patients suffer from an activating or inhibitory disorder of the platelets. More detailed studies, such as platelet aggregometry in patients with leptospirosis, would be an important advance in the field.

Table 1 summarize the major hypotheses to explain leptospirosis-associated disturbances of blood vessels, lungs and hemostasis and the most important Pro and Cons based on current literature.

### 5. Conclusions

Based on both new and old information from the scientific literature, some concepts on the pathogenesis of leptospirosis are rapidly evolving and many fields for research still open. We propose that the involvement of blood vessels in leptospirosis must be comprehended as a sepsis-like diffuse process of endothelial activation/damage rather than a classical systemic vasculitis. Lesions associated with unequivocal damage of the vessel walls are rare and do not represent the dominant mechanism of the target organ pathology. Pulmonary hemorrhages are probably multifactorial and recent evidence does not favor a role of autoimmunity. Further investigation of strain variations, exposure to hydrocarbons and association with renal dysfunction will be a priority to better understand SPHS. Thrombocytopenia is a consistent feature of leptospirosis, but it is not clear whether it is attributable to sepsis-related mechanisms. In addition, further investigation is required to define whether platelet function is activated or inhibited during severe leptospirosis.

As the major impact of leptospirosis depends on the appropriate supportive therapy, the comprehension of the mechanisms leading to hemostatic imbalance would be a major step for the development of new therapeutics, which are urgently needed for the management of severe disease.

### References


