

What role do coagulation disorders play in the pathogenesis of leptospirosis?

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Summary

Leptospirosis is a zoonosis of worldwide distribution, spread by the urine of infected animals. It is a major public health problem, especially in developing countries, where circumstances for transmission are most favourable. The clinical picture varies from mild disease to a severe illness with haemostatic derangements and multiorgan failure eventually leading to death. Although the haemorrhagic complications of severe disease are serious, the pathophysiology is scarcely elucidated. The complex mechanisms involved in inflammation-induced coagulation activation are extensively studied in various infectious diseases, i.e. Gram-negative sepsis. Tissue factor-mediated coagulation activation, impairment of anticoagulant and fibrinolytic pathways in close concert with the cytokine network are thought to be important. But for human leptospirosis, data are limited. Because of the growing interest in this field, the impact of leptospirosis, and the availability of new therapeutic strategies, we reviewed the evidence regarding the role of coagulation in leptospirosis and provide suggestions for future research.

keywords Leptospirosis, Coagulation, Fibrinolysis, Inflammation, Systematic review

Introduction

Leptospirosis is a zoonosis of global importance (Vinetz 2001) caused by infection with pathogenic *Leptospira* species. Transmission occurs where humans come into direct or indirect contact with urine of infected animals (Levett 2001). Due to the longer survival of leptospire in warm and humid conditions, leptospirosis is predominantly common in the (sub)tropics but transmission occurs in both industrialized and developing countries (Bharti *et al.* 2003). The incidence of leptospirosis during outbreaks and in high exposure risk groups is estimated to exceed 100/100 000 persons/year (WHO). However, this incidence is probably heavily underestimated due to the lack of diagnostic tools in endemic areas and the atypical presentation of the disease, resembling many other illnesses, such as dengue and other haemorrhagic fevers (Ko *et al.* 1999; Sanders *et al.* 1999; Levett *et al.* 2000).

The clinical picture of leptospirosis varies from a febrile illness of sudden onset, to a potentially fatal disease complicated by jaundice, renal failure and serious haemorrhages. Pulmonary haemorrhage has become

recognized among the most important manifestation of human leptospirosis and is increasingly reported over the world (Park *et al.* 1989; O'Neil *et al.* 1991; Zaki & Shieh 1996; Trevejo *et al.* 1998; Yersin *et al.* 2000; Wagenaar *et al.* 2004). Other bleeding manifestations include: haematuria, haematemesis, melaena, epistaxis, petechiae, ecchymoses, bleeding from venipuncture sites and subarachnoid bleeding (Theilen *et al.* 2002). Pathologist's findings in autopsies of humans and animals underline the bleeding tendency, and show widespread haemorrhages throughout the body (Guedes e Silva *et al.* 1980; Pereira da Silva *et al.* 1995; Nally *et al.* 2004; Pereira *et al.* 2005).

Although the haemorrhagic potential of leptospirosis was noted by Weil (1886) as early as 1886, its pathophysiology is still not clearly elucidated, particularly regarding the cause and mechanisms of bleeding. Theoretically, bleeding may be the result of a defect in the primary haemostasis or a dysbalance in secondary haemostasis by depletion of coagulation proteins because of enhanced coagulation or by activated fibrinolysis.

Regarding therapy, there is some evidence that antibiotic treatment of leptospirosis may be beneficial, even given in

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late stage of disease (Watt *et al.* 1988). However there is an urge to improve therapy and supportive care, as severe leptospirosis still accounts for many deaths. Novel therapeutic agents intervening with the coagulation and cytokine cascades may be beneficial. Hence, understanding of the pathogenic mechanisms is crucial. In this study, we review current insights on the involvement of abnormal haemostasis in the pathophysiology of leptospirosis. What is the evidence for defects in primary haemostasis and is there any proof for exaggerated coagulation activation and impaired fibrinolysis?

Method

Citations were retrieved from PubMed and MEDLINE databases, and from locally accessible files of the KIT Royal Tropical Institute library, Amsterdam, the Netherlands. The single terms 'Leptospirosis', 'Weil disease', 'Haemostasis', 'Coagulation', 'Fibrinolysis', 'Inflammation', 'Endothelium', 'Thrombocytopenia', 'Coagulation Protein Disorders', 'Disseminated Intravascular Coagulation', 'Blood Coagulation Disorders', were used and combinations of these terms. Titles, abstracts and references were scanned for relevance on the current topic. Both English and German language papers were reviewed.

Clinical features of leptospirosis

The symptoms seen in human leptospirosis are very diverse. Most infections are mild and only a minority of infected patients will seek medical attention. These usually present with a febrile illness and accompanying symptoms may include: chills, headache, myalgia (especially intense calve pain), gastrointestinal complaints and mild haemorrhagic manifestations, such as conjunctival suffusion. Skin symptoms, such as rash are seen less often and may be misdiagnosed as scrub-typhus or viral infections.

The most severe presentation of icteric human leptospirosis, often referred to as Weil's disease, is characterized by jaundice, renal failure, extensive haemorrhage and a high case fatality rate between 5% and 15% (Bharti *et al.* 2003). Icteric leptospirosis occurs between 5% and 10% of all leptospirosis cases (Heath *et al.* 1965) and is thought not to be the result of hepatocellular damage but rather sepsis-related cholestasis (Moseley 1997). Raises in transaminase levels are usually moderate and the liver function will restore to normal during recovery. Thrombocytopenia is often reported but is not directly correlated with a higher incidence of haemorrhage in leptospirosis. However, thrombocytopenia is positive correlated with the development of acute renal failure and the age of the patients (Edwards *et al.* 1982). Thrombocytopenia and

renal failure were found not to be associated with higher mortality in another retrospective study among 60 cases of leptospirosis (Raoult *et al.* 1983). The renal failure seen in leptospirosis is unique because it is hypokalemic and usually non-oliguric (Seguro *et al.* 1990). When oliguria develops nonetheless, it is a significant predictor of mortality (Daher *et al.* 1999). Leptospirosis can also severely affect the lungs. Pulmonary symptoms may include cough, dyspnoea and haemoptysis and may eventually develop into adult respiratory distress syndrome (ARDS) and severe pulmonary haemorrhage syndrome (SPHS). Haemoptysis may not be evident until patients are intubated and therefore clinicians should suspect SPHS in all patients with signs of respiratory distress, also without signs of haemoptysis (McBride *et al.* 2005). Some evidence suggests that an autoimmune process is responsible for the damage to the pulmonary endothelium (Nally *et al.* 2004; McBride *et al.* 2005; Pereira *et al.* 2005). The severity of respiratory disease is not related to the presence of jaundice (Hill & Sanders 1997). Other clinical manifestations of leptospirosis reported include aseptic meningitis, cardiac involvement with ECG alterations and myocarditis and ocular involvement with autoimmune-associated anterior uveitis.

Haemorrhagic syndromes in leptospirosis

Infection-associated activation of the coagulation cascade may lead to a wide spectrum of clinical effects, ranging from clinical insignificant rise in laboratory markers to severe thrombo-haemorrhagic syndromes, such as disseminated intravascular coagulation (DIC), haemolytic uraemic syndrome (HUS), thrombotic thrombocytopenic purpura (TTP) and vasculitis (van Gorp *et al.* 1999). Patients suffering from these disorders may present with bleeding, thrombosis or both. Various haemostatic markers are used to discriminate between these syndromes. Some of these markers are summarized in Table 1.

Is there any proof that leptospirosis patients suffer from thrombo-haemorrhagic complications? Considering pathological findings, vasculitis with endothelial damage and inflammatory infiltrates composed of monocytic cells, plasma cells, histiocytes and neutrophils is thought to be the pathological hallmark in both human and animal leptospirosis (Levett 2001). In addition, petechial haemorrhages are commonly found and may be extensive. Other findings include: pulmonary haemorrhages, intrahepatic cholestasis, hypertrophy and hyperplasia of Kupffer cells, interstitial nephritis, coronary arteritis and haemorrhagic necrosis in skeletal muscles (Higgins & Cousineau 1977; Guedes e Silva *et al.* 1980; Pereira da Silva *et al.* 1995; Levett 2001; Nally *et al.* 2004; Pereira

Table 1 Expected results of screening tests in haemorrhagic disorders

Defect	Platelet		PT	aPTT	Fib	D-dimer
	BT	count				
Thrombocytopenia	↑	↓	N	N	N	N
Thrombocytopathy	↑	N	N	N	N	N
DIC	↑	↓	↑	↑	↓	↑
Factor VII	N	N	↑	N	N	N
Factor XI, IX, VIII	N	N	N	↑	N	N
Mild hepatic disease	N	N	↑	↑	N	N
Severe hepatic disease	↑	↓	↑	↑	N	N
Vasculopathies	↑	N	N	↑	N	N

BT, bleeding time; PT, prothrombin time; aPTT, activated partial thromboplastin time; Fib, fibrinogen; DIC, disseminated intravascular coagulation.

et al. 2005; Yang *et al.* 2006). The intense intra-alveolar haemorrhages seem to be unique for leptospirosis, the morphological features include interstitial inflammatory infiltrates and extravasations of red blood cells from the capillary bed, which are not seen in other capillary leakage syndromes, such as dengue haemorrhagic fever and pulmonary Hanta (Pereira *et al.* 2005).

Whether thrombocytopenia is in any way due to DIC is a topic of ongoing research in the field of leptospirosis. DIC is a potentially fatal syndrome where activation of the coagulation cascade results in microvascular thrombosis together with a macrovascular bleeding tendency because of depletion from blood cells and proteins, including fibrinogen. Fibrin deposition is the result of tissue factor-mediated thrombin formation and simultaneous inhibition of anticoagulant mechanisms, such as the protein C system. In chorus, high levels of plasminogen activator inhibitor type (PAI)-1, a strong inhibitor of fibrinolysis, and the effects of proinflammatory cytokines contribute to enhance fibrin deposition (Levi & ten Cate 1999). This combination of events may lead to multiorgan failure (MOF) and eventually death. DIC can be caused by several stimuli, such as bacteria, viruses and other invading pathogens. Some animal models showed evidence of DIC in leptospirosis-infected guinea pigs (Higgins & Cousineau 1977; Pereira da Silva *et al.* 1995) and one experimental model showed DIC in infected dogs (Navarro & Kociba 1982), while others did not (Nally *et al.* 2004; Yang *et al.* 2006). Unfortunately, large clinical studies related to the association of DIC and leptospirosis has not been reported. In DIC levels of fibrin and its precursor protein fibrinogen, are usually low due to increased consumption. Several reports concerning leptospirosis demonstrated increased levels of plasma fibrinogen. These findings probably reflect properties of

fibrinogen as an acute phase reactant, although some authors attributed this phenomenon to severe tissue damage, vascular endothelial injury or a compensating mechanism by the liver in response to increased consumption (Higgins & Cousineau 1977; Sitprija *et al.* 1980; Lomar 1990; Pereira da Silva *et al.* 1995; Daher de Francesco *et al.* 2002).

Endothelial cell injury and vasculitis are generally accepted as major pathological characteristics of leptospirosis. Vasculitis is a condition characterized by inflammation of the vessel wall with reactive damage to mural structures causing endothelial cell injury. The clinical result may lead to intravascular thrombosis, subsequent organ infarction and dysfunction.

Lung samples of 12 humans, who died from leptospirosis, showed stimulated vascular endothelium marked by swelling of endothelial cells, an increase in pinocytotic vesicles, and giant dense bodies in the cytoplasm of these cells (Nicodemo *et al.* 1997). An experimental guinea pig model of pulmonary haemorrhage showed no signs of systemic vasculitis (Nally *et al.* 2004). Pulmonary endothelial cell blebs were the only observation made. Immunofluorescence showed the presence of IgM, IgG, IgA and C3 along the alveolar basement membranes of the lungs, possibly causing haemorrhage. Other guinea pig models showed significant vascular damage with vascular congestion and detached endothelial cells (Higgins & Cousineau 1977; de Brito *et al.* 1979; Pereira da Silva *et al.* 1995). A marmoset monkey model, infected with *Leptospira interrogans* serovar Copenhageni, showed microscopic patterns of tissue reaction comparable with dose seen in the severe forms of human leptospirosis. Besides intense intra-alveolar haemorrhages, alveolar septal vessels were congested and contained a higher number of megakaryocytes than the controls. The liver showed mild interstitial oedema, vascular congestion and focal necrosis (Pereira *et al.* 2005).

The TTP and HUS are only scarcely reported in leptospirosis and probably do not play a major clinical role. However, the diagnosis may be missed due to lack of diagnostic tools in developing countries, endemic for leptospirosis. The clinical picture of these syndromes are characterized by both thrombocytopenia and microangiopathic haemolytic anaemia without any other clinically apparent cause. Some studies distinguish the two syndromes with predominantly neurological abnormalities without renal impairment in TTP, and renal failure together with minimal or absent neurological symptoms in HUS. Laing *et al.* (1990) described in a case report the association between TTP and leptospirosis. The case concerned a patient presented with progressive neurological deterioration and haemolysis. Postmortem histology

showed the characteristic hyaline thrombi within small vessels of the brain, heart, lung and kidney, a finding not seen in DIC.

Impairment of renal functioning is one of the features of HUS and is a commonly found symptom in leptospirosis. Based on the findings of thrombocytopenia, fragmented red blood cells, reticulocytosis, high serum FDPs and renal failure the diagnosis of HUS in relation to leptospirosis was published as case report (Hanvanich *et al.* 1985). Relative to the frequent occurrence of renal failure in severe disease, the finding of HUS is probably a rare phenomenon.

General aspects of haemostasis and infection-induced coagulation activation

Basically haemostasis is a balanced system of procoagulant and anticoagulant mechanisms, consisting of the following parts: primary haemostasis, coagulation (secondary haemostasis), anticoagulant mechanisms and fibrinolysis. The formation of a haemostatic plug by adhesion and aggregation of platelets in close concert with the endothelium is considered the primary haemostasis. Secondary haemostasis encompasses a series of protease-zymogen reactions necessary to stabilize this haemostatic plug with the formation of fibrin strands. This process is counteracted by anticoagulant mechanisms, including the proteins C and S and the antithrombin (AT)-heparin pathway. Finally, the plug is degraded by plasmin-mediated cleavage of fibrin strands during the process of fibrinolysis.

Coagulation activation and fibrin deposition during inflammation can be seen as an important part of the host defence of the body against, for example infectious organisms, in order to limit the invading antigen and the inflammatory response to a certain area (Levi *et al.* 2004b). In humans, severe infection or sepsis invariably leads to systemic coagulation activation, impairment of anticoagulant mechanisms and inhibited fibrinolysis (Kinasewitz *et al.* 2004; Levi & van der Poll 2004). In the worst case scenario this may lead to DIC, which may besides haemodynamic and metabolic derangements, contribute to MOF (Levi & ten Cate 1999). Membrane components of virtually all micro-organisms are able to induce this syndrome.

The tissue factor pathway is the most important route for activation of the coagulation cascade in DIC (Levi & ten Cate 1999). A number of cells express tissue factor throughout the body (Mann *et al.* 1998), for example, circulating mononuclear cells when stimulated by proinflammatory cytokines (Osterud & Flaegstad 1983; Franco *et al.* 2000). The majority of cells expressing tissue factor are in tissues not in direct contact with blood, but histological tissue factor appears to be present in all blood-

tissue barriers (Camerer *et al.* 1996). When exposed to blood, tissue factor binds to factor VIIa. This complex catalyses the conversion of factor X to Xa which eventually leads to fibrin clot formation.

Inhibitors of coagulation include AT, proteins C and S and tissue factor pathway inhibitor (TFPI). In severe human sepsis AT and proteins C and S plasma levels are markedly reduced (Mesters *et al.* 1996; Kessler *et al.* 1997; Kinasewitz *et al.* 2004). There is some evidence that the function of the TFPI system is impaired in patients with DIC (Creasey *et al.* 1993). Animal models of severe infection show activation of the fibrinolytic system, which is eventually shut off by PAI-1 activity causing a net procoagulant state (Levi *et al.* 1994; Biemond *et al.* 1995, 1997). This is in concert with severe human sepsis, where non-survivors show besides derangements in coagulation activation, a more pronounced suppression of the fibrinolytic system (van Gorp *et al.* 1999; Kinasewitz *et al.* 2004).

Platelets play an important role in inflammation-induced coagulation activation as well. They can be activated directly by endotoxins and proinflammatory mediators, such as platelet-activating factor (PAF; Zimmerman *et al.* 2002; Levi *et al.* 2003). Once activated platelets start expressing P-selectin on the membrane which mediates not only the adherence of platelets to leucocytes and endothelial cells, but also the enhancement of tissue factor expression on mononuclear cells (Shebuski & Kilgore 2002).

There is an increasing body of evidence that supports the concept of an intensive crosstalk between inflammation and coagulation. Activated coagulation proteases have been shown to induce the release of (pro)inflammatory cytokines, whereas some cytokines (Theilen *et al.* 2002) elicit procoagulant effects (Levi *et al.* 2003).

Current insights in the pathogenesis of abnormal haemostasis in leptospirosis

Primary haemostasis

Disorders of primary haemostasis are very common during the course of many infectious diseases. In this regard, thrombocytopenia is a well-documented feature in leptospirosis, with a high incidence. The underlying mechanism of thrombocytopenia is not always clear. It may be the result of decreased thrombopoiesis, increased platelet consumption because of immune or non-immune causes, thrombocytopeny or a combination. Some authors suggested that bone marrow suppression, due to a direct toxic effect of *Leptospira* could be cause the observed thrombocytopenia (Somers *et al.* 2003) or did not rule out the

possibility (Nicodemo *et al.* 1990). Concerning non-immune platelet destruction, one study explored the possibility of platelet consumption because of DIC as an explanation for the frequently observed thrombocytopenia in leptospirosis. Based upon laboratory measurements the authors concluded that there was no causal relationship (Edwards *et al.* 1986). In general, it is assumed that thrombocytopenia is mainly immune-mediated contributing to enhanced clearance. For leptospirosis in this regard, some authors postulated that this phenomenon could be attributed to a yet unknown platelet antibody (Kahn 1982); however, there are technical difficulties in the study of such antibodies (Nicodemo *et al.* 1990). One case report offered some evidence for immune-mediated platelet destruction, as was shown by high titres of surface-bound immunoglobulin, the complement factor C3d and the beneficial response to treatment with methylprednisolone and hydrocortisone (Davenport *et al.* 1989). Several other studies demonstrated peripheral platelet destruction by bone marrow aspirates, revealing hypercellularity and increased megakaryocytes (Davenport *et al.* 1989; Turgut *et al.* 2002). Another study suggested, based on human postmortem lung fragments that the thrombocytopenia was determined by activation, adhesion and aggregation of platelets to stimulated vascular endothelium (Nicodemo *et al.* 1997). Platelet surface receptors with high affinity for subendothelium adhesion glycoproteins (e.g. von Willebrand) may facilitate this process. Indeed, an amorphous substance was found, interposed between the endothelial cells and platelets in places where the subendothelial collagen was not exposed. No fibrin was found in the platelet aggregates. An experimental guinea pig model by Yang *et al.* (2006) showed evidence for platelet activation, reflected by increased plasma levels 11-dehydrogenate thromboxane B2 (11-DH-TXB2) that is considered a sensitive marker. Aggregation of platelets and Kupffer cell phagocytosis of platelets in the liver was another feature found.

Several genes of *L. interrogans* were found to encode proteins with close homology to animal proteins which play an important role in haemostasis (Ren *et al.* 2003), including a protein that resembles the mammalian PAF acetylhydrolase (*pafAH*) and another protein that showed similarity to von Willebrand factor type A domains (*Vwa*). Also an orthologue of paraoxonase (*Pon*) was found, which hydrolyses PAF through its arylesterase activity. It is possible that each of these proteins contribute to haemostatic changes in leptospirosis. In the same study, genes were found encoding for haemolysins and sphingo-myelinase-like proteins. It is not clear whether these proteins play a significant role in the pathogenesis of leptospirosis. Coagulation test results are summarized in Table 2.

Secondary haemostasis

When mononuclear cells were stimulated *in vitro* by a virulent or non-virulent strain of *L. interrogans* serovar Icterohaemorrhagiae coagulant activity was observed, measured by one-stage plasma recalcification time (Miragliotta & Fumarola 1983). There was a significant difference in degree of induction between the virulent and non-virulent strains. Cells incubated with the virulent strain developed significantly higher coagulant activation expressed by shortening of the clotting time, than those cells incubated with the non-virulent strain. Interestingly, there was no coagulation activity observed in factor VII-deficient blood. Based on these data the authors concluded that mononuclear cells induced by (non-)virulent strains of *Leptospira* expressed tissue factor-dependent procoagulant activity. A small Indonesian cohort study revealed activated coagulation, reflected by increased plasma levels of the coagulation activation markers thrombin–antithrombin complex (TAT) and fibrin fragments 1 and 2 (F1 + 2) in severe human subjects (unpublished results). Increased D-dimer plasma levels showed evidence for active fibrinolysis. In contrast, a guinea pig model showed a trend of declining TAT complexes after inoculation of *Leptospira* (Yang *et al.* 2006). This observation is surprising, because TAT is a sensitive marker of coagulation activation.

In Gram-negative sepsis, circulating endotoxins play a pivotal role by activating coagulation via the tissue factor pathway (van Deventer *et al.* 1990; Thijs *et al.* 1993; Baglin 1996; Gando *et al.* 1996). Endotoxins are lipopolysaccharide (LPS) constituents of the outer membrane of Gram-negative micro-organisms. Leptospiral LPS has structural, chemical and immunological properties resembling those of Gram-negative bacterial LPS (Faine *et al.* 1999). Nevertheless, it is relatively non-toxic to cells or animals, but large doses can cause haemorrhages in mice (Faine *et al.* 1999). The possible mechanism of coagulation activation in leptospirosis is illustrated in Figure 1.

Anticoagulation pathways

Upon activation of protein C by the thrombin–thrombomodulin complex activated protein C (APC) is a powerful inhibitor of the coagulation cascade, acting in concert with protein S. APC is believed to be not only a potent anticoagulant, but also an enzyme that modulates a number of inflammatory processes through direct cellular or indirect pathways [the latter through interaction with protease-activated receptors (PARs) or the endothelial protein C receptor (EPCR)]. The nature and extent of these inflammatory actions is the subject of ongoing research. While proinflammatory cytokines may reduce the cellular

Table 2 Results of tests of coagulation

Test and study reference	Mean value	
	Cases	Controls
Platelets		
Daher de Francesco <i>et al.</i> (2002; decreased: $<150 \times 10^3/\text{mm}^3$)	69 (SD 65)	
Edwards <i>et al.</i> (1986; decreased: $<100 \times 10^9/\text{l}$)*	42.3 (SD 29.8)*	207.8 (67.2)
Jaroonvesama <i>et al.</i> (1975; $129\text{--}230 \times 10^3/\text{mm}^3$)	135	
Nicodemo <i>et al.</i> (1990; $150\text{--}450 \times 10^3/\text{mm}^3$)	70	
Edwards <i>et al.</i> (1986; decreased: $\leq 100 \times 10^9/\text{l}$)	125 (SD 84)	
Edwards <i>et al.</i> (1982; decreased: $\leq 100 \times 10^3/\text{mm}^3$)	46.9 (SD 26.7)*	188.2 (SD 69.4)†
Prothrombin time		
Daher de Francesco <i>et al.</i> (2002; 12–14.4 s)	13.3 (SD 0.8)	
Jaroonvesama <i>et al.</i> (1975; 15–16 s)	25.1	
Edwards <i>et al.</i> (1986; no normal values denoted)	13.3 (SD 2.1)	
Edwards <i>et al.</i> (1982; 13–15 s)	15.8 (SD 2)*	15.9 (SD 1.5)†
Sitprijia <i>et al.</i> (1980; 70–100%)	78.9 (SEM 0.9)	
Activated partial thromboplastin time		
Daher de Francesco <i>et al.</i> (2002; 32–38.4 s)	32.7 (SD 2.1)	
Jaroonvesama <i>et al.</i> (1975; 55–69 s)	73	
Edwards <i>et al.</i> (1986; no normal values denoted)	28.6 (SD 12.3)	
Sitprijia <i>et al.</i> (1980; 25–55 s)	33.4 (SEM 0.5)	
Thrombin time		
Daher de Francesco <i>et al.</i> (2002; 9.8–11 s)	11 (SD 1.4)	
Jaroonvesama <i>et al.</i> (1975; 5–6 s)	6.9	
Fibrinogen		
Daher de Francesco <i>et al.</i> (2002; 150–380 mg/dl)	515 (SD 220)	
Jaroonvesama <i>et al.</i> (1975; 306 mg/100 ml)	529	
Sitprijia <i>et al.</i> (1980; 200–400 mg/dl)	818 (SEM 57.6)	
Fibrinogen degradation products (FDP)		
Edwards <i>et al.</i> (1986; $<7.5 \mu\text{g}/\text{ml}$)	8.1 (SD 4.8)*	9.5 (SD 4.4)
Jaroonvesama <i>et al.</i> (1975; 6–9 $\mu\text{g}/\text{ml}$)	12.4	
Sitprijia <i>et al.</i> (1980; $<0.5 \text{ mg}/\text{ml}$)	3.7 (SEM 0.39)	
Factor V		
Jaroonvesama <i>et al.</i> (1975; 130%)	77	
Sitprijia <i>et al.</i> (1980; 70–120%)	90.8 (SEM 0.9)	
Factor VIII		
Jaroonvesama <i>et al.</i> (1975; 120%)	113	
Sitprijia <i>et al.</i> (1980; 70–120%)	88.6 (SEM 1.3)	
Factor X		
Jaroonvesama <i>et al.</i> (1975; 130%)	113	

*All thrombocytopenic patients.

†Non-thrombocytopenic leptospirosis cases.

expression of the cofactors thrombomodulin and EPCR, diminishing the PC mechanism, in infectious disease in general, a second mechanism may undermine this natural anticoagulant system. In patients with severe leptospirosis and significantly elevated concentrations of antiphospholipid antibodies (Rugman *et al.* 1991; Daher de Francesco *et al.* 2002) the function of the protein C system may also be inhibited (Marciniak & Romond 1989). Antithrombin, a circulating serine protease inhibitor, is another important inhibitor of the activated coagulation system. The third anticoagulant pathway consists of TFPI, primarily synthe-

sized in the microvascular endothelium. Its anticoagulant mechanism is due to quaternary complex formation with factor X and tissue factor–factor VII, thereby impairing coagulation. These pathways have never been studied in leptospirosis.

Fibrinolysis

In the circulating blood the process of fibrinolysis is important for limited proteolysis of cross-linked fibrin molecules. Infection-associated coagulation activation is

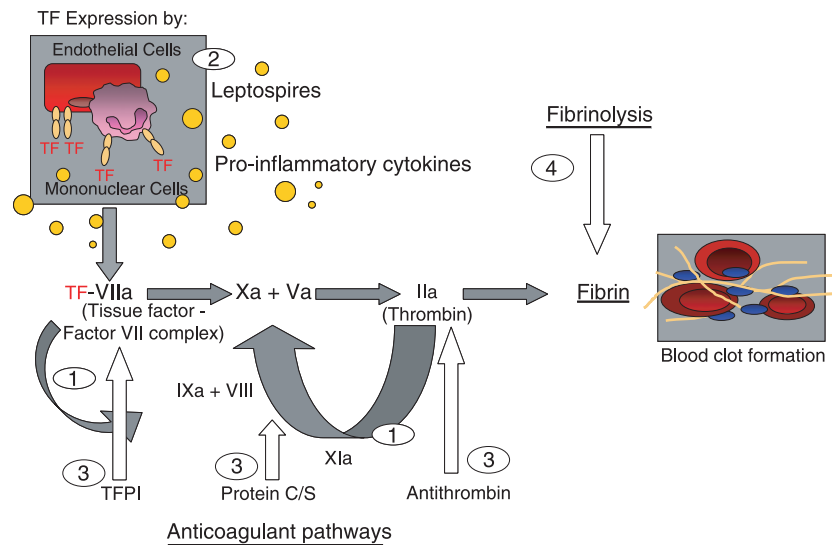


Figure 1 Mechanisms of coagulation activation in leptospirosis. (1) Amplification loops: (a) activation of factor IX by the tissue factor (TF)–factor VIIa complex, generates additional factor Xa; (b) thrombin-induced factor XI activation, leading to additional factors IXa and Xa; (c) activation of the essential co-factors V and VIII by thrombin. (2) Tissue factor expression by monocytes and endothelial cells. Leptospiral lipopolysaccharide or other outer membrane components may induce cytokine release by monocytes and/or endothelial cells. Both may induce TF expression and thereby induce coagulation activation, inhibition of anticoagulant pathways or fibrinolysis. (3) The anticoagulant pathways: proteins C and S, antithrombin and TF pathway inhibitor. (4) The process of fibrinolysis breaks down cross-linked fibrin molecules. Coagulation activation triggers the activation of the fibrinolytic system by increasing levels of tissue plasminogen activator and urokinase plasminogen activator, followed by an increase of plasminogen activator inhibitor type-1. This is a strong inhibitor of the fibrinolysis.

followed by activation of the fibrinolytic system due to increased levels of tissue plasminogen activator (tPA) and urokinase plasminogen activator (uPA), followed by an increase of PAI-1. The degree of rise in systemic PAI-1 concentration determines whether a net procoagulant state occurs, such as in case of DIC (Biemond *et al.* 1995). Only a limited amount of data exists about the activation of the fibrinolytic system in leptospirosis. Elevated levels of fibrin degradation products (FDP) in leptospirosis were reported in a number of studies (Jaroovesama *et al.* 1975; Higgins & Cousineau 1977; Sitprija *et al.* 1980; Navarro & Kociba 1982; Edwards *et al.* 1986; Daher de Francesco *et al.* 2002). There are no studies focusing on regulatory pathways of fibrinolysis, like the PAI-1 protein.

Leptospirosis and the endothelial cell

As mentioned earlier, pathological findings points to the endothelial cell to be important in the pathophysiology of leptospirosis. The endothelial cell plays a crucial role in the regulation of both coagulation and fibrinolysis (Keller *et al.* 2003). Endothelium is able to express tissue factor, von Willebrand factor and a wide variety of cytokines in reaction to various pathogens. Endothelial cells exposed to

a pathogen lose their anticoagulant properties, which results in a net procoagulant state (Keller *et al.* 2003). Increased levels of thrombomodulin found in an animal model, may reflect endothelial cell injury in leptospirosis (Yang *et al.* 2006) and point to soluble thrombomodulin as a marker for endothelial damage. An other experimental model found that intact *Leptospira* and leptospiral peptidoglycans activate cultured human endothelial cells, reflected by an increased adhesiveness for neutrophilic granulocytes (Dobrina *et al.* 1995). This resembles the effects of LPS on endothelial cells.

The complete genomic sequencing of the virulent serovar Lai identified a *cola* gene encoding for microbial collagenase (Ren *et al.* 2003). The authors proposed that collagenase-mediated injury of the vascular endothelium may contribute to the loss of haemostasis in human leptospirosis.

Inflammatory response to *Leptospira*

Cytokines play an important role in the activation of the coagulation cascade (Levi *et al.* 2003). Surface-exposed membrane components, such as LPS, trigger a general host immune response. Thus, far over 260 membrane-associ-

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ated proteins have been identified in *Leptospira*, and for most of these the relevance with regard to an immunogenic reaction remains to be established (Nascimento *et al.* 2004). Six surface-exposed lipoproteins have been identified, of which LipL32 and LipL21 are of most interest, because they are found in all pathogenic *Leptospira* (Cullen *et al.* 2002, 2005). Both leptospiral LPS and LipL32 interact with the Toll-like receptor (TLR)-2 and CD14 to signal to activated macrophages (Werts *et al.* 2001). LPS of Gram-negative bacteria interact predominantly with the TLR4. In contrast, another study found a protective role for the TLR4 in an experimental leptospirosis mouse model (Viriyakosol *et al.* 2006), which suggest this receptor is of importance for leptospiral LPS. Injected tumour necrosis factor (TNF)- α activated the coagulation system via the tissue factor pathway in healthy volunteers (Bauer *et al.* 1989; van der Poll *et al.* 1990; van Deventer *et al.* 1990). Elevated plasma concentrations of TNF- α were found in patients affected by leptospirosis (Estavoyer *et al.* 1991) and higher TNF- α levels were associated with disease severity and mortality (Tajiki & Salomao 1996). Leptospiral peptidoglycans induce TNF- α release from peripheral blood mononuclear cells in a dose-dependant manner *in vitro* (Cinco *et al.* 1996). Other *in vitro* experiments showed that the inflammatory response was also mediated primarily by a Th1 response, involved in cellular immunity. Increased levels of interferon (IFN)- γ , interleukin (IL)-12p40, IL-12 and TNF- α were found after stimulation with heat-killed *Leptospira* (de Fost *et al.* 2003; Klimpel *et al.* 2003). *Leptospira interrogans* glycolipoprotein (GLP) was found to induce cellular production of TNF- α and IL-10 in peripheral blood mononuclear cell cultured from healthy donors (Diament *et al.* 2002).

Conclusion and discussion

Leptospirosis is one of the world's most prevalent zoonoses, with a clinical picture varying from mild to potentially life-threatening disease, in which haemostatic derangements play a central role. Despite these facts leptospirosis is a neglected disease, which explains that many crucial aspects concerning its pathogenesis remain unanswered. Besides antibiotic therapy, which is the cornerstone of treatment, we urgently need to improve supportive treatment, especially for cases with life-threatening bleeding complications.

From the studies reviewed we may also conclude that in leptospirosis, as in other infectious disease, the bleeding tendency is the result of a dysbalance in the haemostatic equilibrium, although it is unclear how this dysbalance is triggered and what inflammatory and coagulation proteins are involved. The haemostatic dysbalance may lead to DIC,

but no human studies with modern, sensitive assays have been performed to elucidate this question. DIC is a clinical syndrome, and only a combination of laboratory markers can establish or rule out the diagnosis (Levi & ten Cate 1999). Ascertain such a syndrome is important to assess the potential role of new treatment options available in this regard.

It would be premature to speculate what is the origin of the bleeding. However, in several studies the endothelial cell seems to be one of the target cells in leptospirosis. After infection the endothelial cell may lose its antithrombotic properties and we hypothesize that this might well be the link to an out-of-balance coagulation cascade. The endothelial cell may influence haemostasis due to stimulation of cytokines in concert with, e.g. circulating lymphocytes or platelets or by direct effects of the invading micro-organism, i.e. *Leptospira*. Ample evidence stress the importance of the endothelium as the conductor of the orchestra of procoagulant and anticoagulant pathways (Levi *et al.* 2002; Keller *et al.* 2003).

To understand the haemorrhagic complications of leptospirosis, there is an urgent need for prospective studies. Efforts should be made to enrol both mild and severe cases in all stages of disease, using case record forms to be able to relate clinical signs and clinical outcome with laboratory disturbances. Sensitive laboratory tests available, focusing on the different involved pathways should be used. Studies should address the role of the endothelium because its possible central role in the pathogenesis of leptospirosis as posed here. Emphasis should be put on identifying involved (pro)inflammatory cytokines, given the intensive crosstalk between coagulation and inflammation. Finally, contributing factors-like age, gender and genetic profile should be taken into account. Such studies will provide us the data necessary to improve supportive and therapeutic management strategies to treat this potentially fatal disease.

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Quel rôle ont les troubles de la coagulation dans la pathogénie de la leptospirose?

La leptospirose est une zoonose rencontrée à l'échelle mondiale, propagée par l'urine des animaux infectés. C'est un problème majeur de santé publique, particulièrement dans les pays en voie de développement où les circonstances pour la transmission sont les plus favorables. L'aspect clinique varie d'une maladie bénigne à une maladie sévère avec des troubles hémostatiques et une déficience de plusieurs organes menant au décès. Bien que les complications hémorragiques de la maladie sévère soient sérieuses, la pathophysiologie est à peine élucidée. Les mécanismes complexes impliqués dans l'activation de la coagulation induite par l'inflammation ont été intensivement étudiés dans diverses maladies infectieuses, i.e. sepsis à gram négatif. L'activation de la coagulation médiée par le facteur du tissu, les déficiences de l'anticoagulant et les voies métaboliques fibrinolytiques en étroite corrélation avec le réseau des cytokines semblent être importants. Mais pour la leptospirose humaine, les données demeurent limitées. En raison de l'intérêt croissant pour ce domaine, l'impact de la leptospirose et la disponibilité de nouvelles stratégies thérapeutiques, nous avons passé en revue l'évidence concernant le rôle de la coagulation dans la leptospirose et fournissons des suggestions pour la recherche future.

mots-clés: Leptospirose, coagulation, fibrinolyse, inflammation, revue systématique

‘Qué papel juegan los desórdenes de coagulación en la patogénesis de la leptoespirosis?’

La leptoespirosis es una zoonosis mundialmente distribuida, diseminada por la orina de animales infectados. Es un importante problema de salud pública, especialmente en países en vías de desarrollo, en donde las circunstancias de transmisión son las más favorables. El cuadro clínico varía desde la enfermedad leve a la severa, con trastornos hemostáticos y fallo multiorgánico siendo eventualmente letal. Aunque las complicaciones hemorrágicas de la enfermedad severa son serias, la patofisiología raramente es elucidada. Se han estudiado extensivamente los complejos mecanismos involucrados en la activación de la coagulación inducida por la inflamación en varias enfermedades infecciosas, como es el caso de la sepsis por gram negativos. Se cree que son importantes la activación de la coagulación mediada por factores tisulares, la alteración de las vías fibrinolítica y anticoaguladora, conjuntamente con la alteración de la red de citocinas. Pero en el caso de la leptoespirosis humana, los datos siguen siendo limitados. Debido al creciente interés en esta área, el impacto de la leptoespirosis y la disponibilidad de nuevas estrategias terapéuticas, hemos revisado la evidencia con respecto al papel de la coagulación en la leptoespirosis y damos sugerencias para futuros estudios.

palabras clave: Leptoespirosis, Coagulación, Fibrinólisis, Inflamación, Revisión sistemática

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