

## Review Article

### Review on severity markers in leptospirosis

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**Abstract.** Leptospirosis is a zoonotic disease caused by the pathogenic strains of *Leptospira*. Outbreaks of leptospirosis have been reported following water sports events and floods particularly in men. The symptoms range from mild acute febrile illness to severe form with multi organ failure. Severe leptospirosis increases the likelihood of mortality and may require medical interventions in the form of dialysis and/or mechanical ventilation. It is important to predict severe leptospirosis to optimize medical care since non-severe patients reported to progress to severe form during the immune phase of the illness. The exaggerated host immune response causing endothelial and organ damages was shown to be associated with disease severity and mortality. This review presents the association of immune and endothelial activation markers, biochemical and genetic markers with disease severity in leptospirosis.

#### INTRODUCTION

Leptospirosis is a waterborne zoonotic disease caused by the pathogenic strain of *Leptospira*, *L. interrogans* (Levett, 2001). It occurs throughout the world and the incidence rate range from 0.1 to 1 per 100,000 inhabitants in temperate regions and 10 to 100 per 100,000 inhabitants in tropical regions (WHO, 2013). Several countries including Malaysia, Sri Lanka, Philippines, Germany, and Austria have reported outbreaks of leptospirosis in the recent years (Agampodi *et al.*, 2009; Brockmann *et al.*, 2010; McCurry 2009; Radl *et al.*, 2011; Yuzsniayati *et al.*, 2015). Initial symptoms of leptospirosis include acute fever, myalgia, nausea, skin rash, chills and headache similar to other acute febrile illnesses and these symptoms can resolve spontaneously (Tubiana *et al.*, 2013). However, about 5-15% of patients with the mild disease will progress to the severe form known as Weil's syndrome during their immune phase which includes multiorgan failure with hemorrhages,

splenomegaly, tubulointerstitial nephritis, jaundice, pulmonary damages, and septic shock (Mikulski *et al.*, 2015). Patients with leptospirosis are categorized as severe or non-severe cases based on the operational criteria. Severe cases are those who died or required dialysis and/or mechanical ventilation for severe pulmonary hemorrhage syndrome (SPHS) while non-severe cases are those who survived with neither dialysis nor mechanical ventilation during their hospital stay (Herrmann-Storck *et al.*, 2010; Mikulski *et al.*, 2015). Some of the factors that may lead mild disease progressing the severe form are smoking, serovar of infecting *L. interrogans*, infectious dose, difference in host immune response and delay in antibiotic therapy (Reis *et al.*, 2013; Tubiana *et al.*, 2013). Initiation of antimicrobial treatment itself can trigger a febrile inflammatory response called Jarisch-Herxheimer reaction (JHR) that requires hemodynamic support (Guerrier *et al.*, 2013a). The mortality rate for severe leptospirosis patients has been reported to be 5% to 10% and it can rise up

to 50% in patients with SPHS (Gouveia *et al.*, 2008). Thus, early prediction of severe leptospirosis is very important to optimize medical care since there are reports on the deterioration of non-severe patients requiring dialysis, mechanical ventilation, and transfusion of platelets during the later phase of their illness (Mikulski *et al.*, 2015).

Although the mechanism of pathogenicity of leptospirosis is not fully understood, evidence show that exaggerated host immune response could play a role in disease severity (Abdulkadar *et al.*, 2002; Gouveia *et al.*, 2008; Rizvi *et al.*, 2014). It has been demonstrated that the initial delay of leptospira elimination in some patients may provide time for bacterial dissemination to various organs and fatality might relate to failure to mount robust T cell and B cell responses during acute phase due to the defect in antigen presentation (Lindow *et al.*, 2016). The inflammatory process resulting from the activation of immune response particularly the cytokine storm that occurs during the clearance of *Leptospira* from the circulation is shown to be associated with organ damage observed in patients with severe clinical manifestations (Chirataworn and Kongpan, 2014; Guerrier *et al.*, 2013b; Kyriakidis *et al.*, 2011; Reis *et al.*, 2013; Tajiki *et al.*, 1997). Furthermore, bleeding associated with endothelial activation and changes in the levels of biochemical constituents due to multiorgan failure was reported in severe disease (Chang *et al.*, 2005; Goeijenbier *et al.*, 2015). Certain HLA types and gene polymorphisms were also reported to be associated with the development of severe leptospirosis (Cedola *et al.*, 2015). Thus, levels of inflammatory mediators such as cytokines, chemokines, adhesion molecules and serum proteins may serve as indicators to monitor disease progression (Chirataworn and Kongpan 2014; Goeijenbier *et al.*, 2015).

## I. IMMUNE ACTIVATION MARKERS

Evidence indicate that the host immune response against leptospirosis involves both humoral and cell mediated immunity (Blasi

*et al.*, 2007; Gaudart *et al.*, 2008; Klimpel *et al.*, 2003; Silva *et al.*, 1995). *Leptospira* components including glycolipoprotein (GLP) and lipopolysaccharide (LPS) released after bacterial lysis causes inhibition of Na/K-ATPase that may directly cause tissue injury or increased plasma levels of nonesterified fatty acid (NEFA) concentrations can stimulate the production of inflammatory mediators leading to exacerbation of the immune response associated with multiorgan dysfunction observed in severe disease (Diament *et al.*, 2002; Dorigatti *et al.*, 2005; Goncalves-de-Albuquerque *et al.*, 2012). It has been shown that outer membrane proteins (OMPs) of leptospire induces dendritic cell (DC) maturation and activation through TLR-2 (Guadart *et al.*, 2008; Werts *et al.*, 2001). Microglial cells, when exposed to *Leptospira* antigens, show NF $\kappa$ B activation and p38 phosphorylation resulting in cytokine release (Blasi *et al.*, 2007).

### i. Cells of the immune system

Analysis of circulating immune cell counts between leptospirosis patients and controls showed that the total white cells and neutrophils were higher in severe leptospirosis patient with the presence of neutrophilia at the early stage of illness (Lindow *et al.*, 2016; Raffray *et al.*, 2015). In contrast, the number of monocytes, lymphocytes, gamma/delta T cells and platelets counts were significantly decreased (Abgueguen *et al.*, 2008; Crouzet *et al.*, 2011; Goeijenbier *et al.*, 2015; Lindow *et al.*, 2016; Raffray *et al.*, 2015; Reis *et al.*, 2013; Spichler *et al.*, 2008; Tubiana *et al.*, 2013). The number of IL-2, IFN- $\gamma$  and TNF- $\alpha$  producing CD4<sup>+</sup> T cells after stimulation with *Leptospira* antigens was highest in patients with severe and life threatening symptoms (Volz *et al.*, 2015). Detailed flow cytometry studies are required to better understand the involvement of various cell types in disease pathogenesis in severe leptospirosis.

### ii. Antibodies

Humoral-immune response is the major immune system that helps to eliminate *Leptospira* from the host (Adler *et al.*, 1980).

Most of the anti-leptospiral antibodies produced are against the OMP of the pathogen as they are the earliest antigenic structures encountered and recognized by the plasma cells (Farely *et al.*, 1987). Leptospira-specific IgM could be detected after five or seven days of symptomatic illness and its titer drops sharply after the acute stage (Chapman *et al.*, 1991). Leptospira-specific IgG is usually detected during the second week and high levels of this antibody can be retained for several months or even years (Picardeau *et al.*, 2014; Silva *et al.*, 1995). The early detection of both anti-leptospiral IgM and IgG antibodies is an indicator of reinfection with a different serovar of Leptospira (Abdulkader *et al.*, 2002). It has been reported that older patients with increased leptospira-specific IgG and IgA antibody titers experienced worse pulmonary and renal function and fever for a longer period compared to patients with low antibody titers showing severity could be associated with the intensity of humoral immune response (Abdulkader *et al.*, 2002). It is suggested that the increased intensity of immune activation could be due to previous Leptospira infection and presence of specific antibodies might worsen the tissue damage as the sub-agglutinating amounts of antiserum was shown to enhance attachment of virulent leptospire to the cells *in vitro* (Faine *et al.*, 1999). In addition, presence of anticardiolipin antibodies has been reported in leptospirosis patients with severe complications showing involvement of non-specific humoral immune response in severity of the disease (Rugman *et al.*, 1991). A recent study shows that the agglutinating anti-leptospira antibody titers were lower in nonsurvivors that correlated with less abundance of immunoglobulin transcripts during early acute infection (Lindow *et al.*, 2016).

### iii. Cytokine and chemokines

Cytokines play an important role in inflammatory reactions and levels of certain inflammatory cytokines are shown to predict the disease outcome (Tajiki *et al.*, 1997). It has been demonstrated that Leptospira antigens were able to induce the production

of inflammatory cytokines in *in vitro* studies and in animal models (Blasi *et al.*, 2007; Naiman *et al.*, 2001). Human peripheral blood mononuclear cells (PBMCs) shown to release TNF- $\alpha$  and IL-6 when incubated with components of leptospira (Cinco *et al.*, 1996). Upregulation of IL-8 has been shown in murine proximal tubule cells (PTC) following tubule-interstitial nephritis initiated by the leptospiral OMPs (Hung *et al.*, 2006). Hemolysins of Leptospira were shown to induce the production of TNF- $\alpha$ , IL-1 $\beta$  and IL-6 in human and murine macrophages (Wang *et al.*, 2012). Expression of TNF- $\alpha$  and IL-10 mRNA was observed three and five days post Leptospira infection respectively in hamsters (Lowanitchapat *et al.*, 2010). TNF- $\alpha$ , IL-10, IL-1 $\beta$  and cyclooxygenase-2 gene expression was significantly higher in dead hamsters infected with leptospire than in the survivors (Vernel-Pauillac *et al.*, 2010).

Leptospirosis infected patient show elevated level of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, and IL-10 compared to healthy individuals (Chirataworn *et al.*, 2016; Reis *et al.*, 2013). The levels of some these cytokines were found to be higher in patients with severe symptoms with fatal outcome compared to mild cases (Balamayooran *et al.*, 2010; Kyriakidis *et al.*, 2011; Reis *et al.*, 2013; Rizvi *et al.*, 2014; Tajiki and Salomao, 1996). Higher levels of these cytokines were shown to be associated with disease pathogenesis such as lung injury in SPHS, hepatitis and hence considered as predictors of mortality (Kyriakidis *et al.*, 2011; Reis *et al.*, 2013; Rizvi *et al.*, 2014). High IL-10/TNF- $\alpha$  ratio was shown to be associated with severity and mortality although contradictory reports exist (Kyriakidis *et al.*, 2011; Miluski *et al.*, 2015; Tajkiki *et al.*, 1997).

Leptospira lipoprotein extracts were shown to stimulate the production of chemokines CCL2/MCP-1 and CXCL2/MIP-2 in an *in vitro* experiment (Hung *et al.*, 2006). Increased expression of Interferon- $\gamma$ -inducible protein-10 (IP-10)/CXCL10 and MIP-1 $\alpha$ /CCL3 has been found in infected organs of hamsters (Lowanitchapat *et al.*, 2010; Matsui *et al.*, 2012). Sustained expression of CCL2/MCP-1 and CXCL1/KC in the lungs was shown to correlate with

disease severity in susceptible C3H/HeJ mice (da Silva *et al.*, 2009; Da Silva *et al.*, 2012). Levels of IP-10/CXCL10 and Mig/CXCL9 was shown to be higher in leptospirosis patients at the early stages of illness than in controls showing activation of cellular immune response (de Fost *et al.*, 2007). The level of IP-10 was significantly higher in patients with severe disease compared to mild disease (Papa *et al.*, 2015). RANTES(CCL5) serum levels are suggested as a diagnostic marker to identify patients at risk for developing severe disease (Lindow *et al.*, 2016).

#### **iv. Other serum inflammatory mediators related to severe leptospirosis**

It has been demonstrated that leptospirosis patients who exhibited SPHS found to have an elevated level of tissue damage marker sST2, the IL-1 receptor like -1 protein (IL1RL1) in their plasma. Soluble ST2 levels are associated with bleeding in patients with severe leptospirosis (Wagenaar *et al.*, 2009). Pentraxin 3 (PTX 3) is a mediator of inflammation which is secreted by various cells such as mononuclear phagocytes, dendritic cells, fibroblasts, endothelial cells and epithelial cells (de Kruijff *et al.*, 2010; Mauri *et al.*, 2008). Increased levels of PTX3 in leptospirosis patients were reported to be associated with severity and mortality and PTX3 was noted as a more sensitive acute phase protein than C-reactive protein (CRP) (Mikulski *et al.*, 2015; Wagenaar *et al.*, 2009). Higher level of MBL (>1000 ng/mL), a component of the complement system was shown in the serum of leptospirosis patients with more severe complications compared to control group (Miranda *et al.*, 2009). The level of immune activation markers that are related to endothelial cell activation such as soluble interleukin 2 receptor (sIL-2r) and soluble Fas ligand (sFasL) were shown to be associated with mortality, bleeding and increased duration of hospital stay in leptospirosis patients (Goeijenbier *et al.*, 2015; Petro *et al.*, 2000). An in-depth characterization of gene expression in Brazilian patients showed 389 unique transcripts associated with fatality. Fatal cases exhibited decreased transcription of

genes involved in chemotaxis, coagulation and adaptive immune responses particularly the antimicrobial peptide, cathelicidin which is an important molecule that protects against fatal leptospirosis (Lindow *et al.*, 2016).

## **II. ENDOTHELIAL ACTIVATION MARKERS**

Respiratory failure and acute lung injury (ALI) in leptospirosis are characterized by cytokine release, loss of endothelial integrity, increased permeability, protein extravasation and edema. Patients with severe leptospirosis generally suffer extensive pulmonary hemorrhage caused by the inhibition of Na/K pump that might contribute to lung failure (Goncalves-de-Albuquerque *et al.*, 2012). Recombinant OMPs of pathogenic leptospira have been shown to activate HUVEC cells and induce upregulation of adhesion molecules such as intercellular cell adhesion molecule-1 (ICAM-1) and E-selectin on their surface in an *in vitro* assay (Atzingen *et al.*, 2009; Vieira *et al.*, 2007). The concentration of soluble E-selectin (sE-selectin) was found to be elevated in plasma of patients with severe leptospirosis with mortality (Goeijenbier *et al.*, 2015). The expression of ICAM-1, VCAM-1 and C3a receptor promotes leukocyte recruitment to infected tissues and increased expression of these adhesion molecules were reported on alveolar septa of patients died from leptospirosis with pulmonary involvement (Bernardi *et al.*, 2012). Angiopoietin-2, asymmetric dimethyl arginine (ADMA) and its isomer, symmetric dimethylarginine (SDMA) are markers for endothelial activation and increased concentrations of these components have been observed in patients during the complicated course of leptospirosis with renal failure (Lukasz *et al.*, 2014). Von Willebrand factor (vWF) is a component of coagulation system that mediates adhesion of platelets and its concentration was found to be highly increased among non-survivors with severe leptospirosis (Goeijenbier *et al.*, 2015). The level of vascular endothelial growth factor (VEGF) expression was also

significantly higher in patients with severe disease compared to mild disease (Papa *et al.*, 2015).

### III. BIOCHEMICAL MARKERS

The severe form of leptospirosis involves multiorgan failure with hemorrhage resulting in changes in certain biochemical constituents. Leptospirosis patients who develop icteric forms show high aspartate transaminase (AST) and alanine amino transaminase (ALT) levels (Raffray *et al.*, 2015). The AST/ALT ratio was shown to be higher in patients with severe leptospirosis and those with the value more than 3.0 had the high probability of fatal outcome (Chang *et al.*, 2005; Mikulski *et al.*, 2015). Elevated level of bilirubin which is the waste product of heme catabolism was reported in severe leptospirosis patients who showed acute fever with hepatic malfunction and jaundice (Abgueguen *et al.*, 2008; Kalugalage *et al.*, 2013; Mikulski *et al.*, 2015; Spichler *et al.*, 2008). The kidney is an important target organ in leptospirosis and renal impairment in Weil's disease patients is usually associated with increased creatinine concentration and higher levels are shown to be associated with fatality (Cavoli *et al.*, 2013; Crouzet *et al.*, 2011; Raffray *et al.*, 2015; Reis *et al.*, 2013; Spichler *et al.*, 2008; Tubiana *et al.*, 2013). Patients with severe bleeding and inflammation of the kidneys due to leptospirosis showed decreased levels of glutathione (GSH) and its levels tend to be negatively correlated with serum creatinine concentration (Aroujo *et al.*, 2014). Patients with acute and severe leptospirosis show higher levels of nitric oxide compared to healthy individuals (Gunaratna *et al.*, 2012; Maciel *et al.*, 2006). Since excretion of nitric oxide in urine is diminished during renal impairment after corrected with creatinine concentration, decreased levels of nitrite was shown to be associated with the severe form of leptospirosis (Kalugalage *et al.*, 2013; Mackenzie *et al.*, 1996). Copeptin is a 39-amino acid peptide released from arginine vasopressin (AVP) during AVP processing and increased levels of copeptin was shown to

be associated with severe leptospirosis (Limper *et al.*, 2010; Petros *et al.*, 2009). Lastly, increased levels of C-reactive protein (CRP) and procalcitonin (PCT) were reported to be associated with severe form of leptospirosis and PCT has been considered as a more discriminatory biomarker for severity compared to CRP (Crouzet *et al.*, 2011).

### IV. GENETIC MARKERS

Few studies have addressed the association between HLA types and gene polymorphisms with susceptibility to leptospirosis. An association between HLA-A (\*24, \*31), HLA-B\*08 and HLA-DQ6 positivity and increased risk to *Leptospira* infection has been reported in Portugal and Illinois, America (Fialho *et al.*, 2009; Lingappa *et al.*, 2004). Single nucleotide polymorphism in IL-1 $\beta$ , IL-2R $\beta$ 1, IL-4, IL-4R $\alpha$  and multiple cytokine inducible SH2-containing protein (CISH) genes were also shown to be involved in susceptibility to *Leptospira* infection (Esteves *et al.*, 2014; Fialho *et al.*, 2009). Recently, it was reported that TLR-2 Arg753Gln and TLR1 Ile602Ser gene polymorphism influenced the risk of developing severe leptospirosis with hepatic insufficiency and jaundice (Cedola *et al.*, 2015). Further investigations are necessary to find the association between genetic polymorphisms and excessive inflammatory response leading to extensive tissue damage in severe leptospirosis.

### CONCLUSION

Distinguishing patients with leptospirosis according to the urgency of their need for care is necessary particularly during an outbreak due to limited medical resources. A number of laboratory parameters can be used for monitoring disease progression in leptospirosis patients. The summary Table of our review indicates that cellular profile particularly neutrophilia and thrombocytopenia in combination with biochemical profiles including levels of AST-ALT, bilirubin, creatinine and CRP that

Table. Severity markers in leptospirosis

Class	Biomarker	Change	Reference
1. Immune activation markers	Cells	Leukocytes	Raffray <i>et al.</i> , 2015; Lindow, 2016
		Neutrophils	
		Monocytes	
		Lymphocytes	
		$\gamma/\delta$ T cells	
		Platelets	
	Antibodies	IgG & IgA	Abdulkadar <i>et al.</i> , 2002
	Cytokines	TNF- $\alpha$	Cinco, 1996; Balamayoran <i>et al.</i> , 2010; Lowanitchapat <i>et al.</i> , 2010; Tajiki <i>et al.</i> , 1997; Vernel-Pauillac <i>et al.</i> , 2010; Wang <i>et al.</i> , 2012 Kyriakidis <i>et al.</i> , 2011; Reis <i>et al.</i> , 2013; Chirataworn <i>et al.</i> , 2016; Hung <i>et al.</i> , 2006; Kyriakidis <i>et al.</i> , 2011
IL-1 $\beta$			
IL-6			
IL-8			
IL-10			
IL-10/TNF- $\alpha$ ratio			
	Chemokines	(IP-10)/CXCL10	de Fost <i>et al.</i> , 2007; Papa <i>et al.</i> , 2015
		Mig/CXCL9	
	Other inflammatory mediators	sST2	Wagenaar <i>et al.</i> , 2009 Wagenaar <i>et al.</i> , 2009; Mikulski <i>et al.</i> , 2015; Miranda <i>et al.</i> , 2009 Petro <i>et al.</i> 2000; Goeijenbier <i>et al.</i> , 2015, Lindow <i>et al.</i> , 2016
		Pentraxin 3 (PTX 3)	
		MBL	
		sIL-2r	
		sFasL	
		Cathelicidin	

<b>II. Endothelial activation markers</b>	Cell surface adhesion molecules Mediators of endothelial function Coagulation components Permeability mediators	sE-selection, ICAM-1, VCAM-1 Angiopoiitin-2 Asymmetric dimethylarginine (ADMA) Symmetric dimethylarginine (SDMA) von Willebrand factor VEGF	Increase Increase Increase } Increase } Increase } Increase } Increase	Goeijenbier <i>et al.</i> , 2015 De carlo Bernardi <i>et al.</i> , 2012 Lukasz <i>et al.</i> , 2014 Goeijenbier <i>et al.</i> , 2015 Papa <i>et al.</i> , 2015
<b>III. Biochemical markers</b>	Liver Enzymes Other soluble substances	AST, ALT, AST/ALT ratio Bilirubin Creatinin Glutathione (GSH) Nitric oxide Copeptin CRP Procalcitonin	Increase Increase Increase Decrease Increase Increase Increase } Increase }	Chang <i>et al.</i> , 2005; Mikulski <i>et al.</i> , 2015; Abgueguen <i>et al.</i> , 2008; Spichler <i>et al.</i> , 2008; Kalugalage <i>et al.</i> , 2013; Mikulski <i>et al.</i> , 2015; Spichler <i>et al.</i> , 2008; Crouzet <i>et al.</i> , 2011; Cavoli <i>et al.</i> , 2013; Tubiana <i>et al.</i> , 2013; Aroujo <i>et al.</i> , 2014 Maciel <i>et al.</i> , 2006; Gunaratna <i>et al.</i> , 2012 Petros <i>et al.</i> , 2009; Limper <i>et al.</i> , 2010 Crouzet <i>et al.</i> , 2011
<b>IV. Genetic markers</b>	Gene polymorphisms	TLR1 TLR2	Ile602Ser Arg753Gln }	Cedola <i>et al.</i> , 2015

are routinely done might help to predict severe disease. Estimation of inflammatory cytokines, endothelial damage markers and identifying HLA types and genetic polymorphism in patients is beyond the scope of many clinical laboratories at this moment and carried out only for research purposes. With the evolution of newer molecular and engineering technologies, laboratory parameters for predicting leptospirosis severity can be expanded to a wider spectrum for better patient management. Immunomodulatory therapy with gluco-corticosteroids and cathelicidin is suggested for patients with severe symptoms with organ failure (Lindow *et al.*, 2016; Volz *et al.*, 2015).

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