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Diagnosing and predicting outcome in leptospirosis: the need for clinically relevant basic sciences research

Leptospirosis is a zoonosis of global distribution, caused by infection with pathogenic *Leptospira* species [1]. The incidence of leptospirosis has been increasing worldwide [2,3]. One of the largest outbreaks in Sri Lanka occurred in 2008, with 7406 reported cases and 204 deaths, giving an incidence rate of 35.7 per 100,000 population. Although many domestic and wild mammals serve as reservoir hosts, human hosts commonly acquire the organism through skin abrasions and mucosal surfaces following contact with water contaminated with the urine of rodents. Traditionally a disease of farmers, in recent years non-traditional exposure has been gaining prominence, in particular recreational exposure [4]. Leptospirosis remains a neglected disease. Despite case fatality rates being higher than with dengue, the demographic distribution of the disease results in leptospirosis deaths having a smaller impact from a public awareness perspective. Apart from antibiotics, there are currently no specific therapies for severe leptospirosis, and management is mainly supportive; corticosteroids are of no proven benefit [5].

Diagnosis of leptospirosis remains a challenge for the treating clinician, especially in areas where the incidence of other acute febrile illnesses is high. The reference standard for laboratory confirmation is the microscopic agglutination test (MAT), which is primarily available at the Medical Research Institute (MRI), Colombo. Despite being the immunological reference standard, MAT has inherent flaws. MAT detects both IgG and IgM antibodies. There is currently little evidence as to the period for which IgG antibodies persist after acute infection. Nor is there clear data on sero-prevalence in the country, and thus there is no clear cut-off for a single MAT titre. While the WHO recommends a titre of >100, it is likely that the cut off titre for acute infection in Sri Lanka is higher, at >400 or even >800. A four-fold rise between acute and convalescent samples is likely to be diagnostic, but is of little value to the clinician treating a suspected case of acute leptospirosis. Genomic tests for diagnosis are likely to be more specific, but are often not sensitive enough, and there is no validated test which is currently widely used. Thus, there has been considerable interest in other rapid immunological tests, such as IgM ELISA, and immunochromatographic tests. There is a growing body of evidence that these tests maybe as reliable, and possibly more useful for early and rapid diagnosis. One of the problems in determining the validity of these new diagnostics is that these tests are often evaluated against MAT as the reference standard. It is the subject of much debate that this model of evaluation of novel diagnostics is flawed [6]. Statistical models which assume that all diagnostic tests are imperfect, for example Bayesian latent class modeling, has been proposed as a better method for evaluating the validity and usefulness of these new diagnostics. There is also a need to determine the

sero-prevalence of leptospirosis in different parts of the country, including type-specific sero-prevalence.

Clinical manifestations of leptospirosis vary considerably. While some patients develop a simple uncomplicated febrile illness which recovers uneventfully, others go on to develop organ dysfunction, predo-minantly acute kidney injury, also pulmonary involvement, liver damage and myocarditis. Organ dysfunction can be severe, and result in fatalities, in particular due to pulmonary haemorrhage and myocarditis. The patho-genesis of severe disease is poorly understood, but it is thought to be largely due to a form of vasculitis. At the point of initial presentation, it is difficult to predict which patients will develop severe disease. If this were possible, clinicians would be able to prioritize healthcare resources better, in particular by identifying patients needing ICU care or dialysis early. While a few clinical features, such as leukocytosis, arrhythmias, thrombocytopenia, and prior alcohol use have been noted to be associated with severe disease, there are as yet no convincing clinical features or scoring systems which identify patients likely to develop severe disease [7,8]. Scoring systems to predict outcome have generated great interest, and one such scoring system which was proposed for patients with severe sepsis is the 'PIRO' (predisposition, infection, host response, organ dysfunction) system, which was modeled on the TNM (tumour, nodes, metastases) classification for cancer. Despite many studies, a robust PIRO system for sepsis has not been developed. While a similar model could potentially be applied to leptospirosis, the main difficulty in developing such a scoring system is the lack of clinical features or other markers which predict severe disease [8].

There has been much interest of late in the use of immunological and biochemical markers which could predict severity in disease, and this holds true for leptospirosis as well. In a proposed PIRO model, these markers would come under the category of indicators of host 'Response'. Although often considered to be similar to sepsis, the pathogenesis of leptospirosis is distinctly different from that of bacterial sepsis. While severe sepsis results from a cytokine storm, with imbalance between pro-coagulant and anti-coagulant pathways, leptospirosis is primarily a vasculitis, although a cytokine storm also probably takes place in severe disease. In both conditions organ damage occurs, but leptospirosis predominantly targets certain organs like the kidneys, the liver and the heart. The deranged immune response in leptospirosis comprises activation of both cell mediated and humoral immunity, although research on this is very limited. Markers of cell mediated immunity such as interferon [IFN]-gamma-inducible protein-10, granzyme B, and monokine induced by IFN-gamma have been shown to be elevated in patients with leptospirosis [9]. Cytokines are likely to play an important role in the pathogenesis of leptospirosis, however their role as predictors of severity is largely unknown. Raised cytokine levels have been demonstrated in severe leptospirosis, however whether these simply reflect the effect of immune activation in general, which is typically seen in any severe illness with organ dys-function, is also not known. Several inflammatory mediators such as serum sST2 levels, cytokines IL-6, IL-8, long pentraxin and copeptin have been shown, in small studies, to be elevated in patients with severe disease [10-12].

One substance that has been studied to some extent in this regard is nitric oxide (NO). It is known that in a state of inflammation, release of inflammatory cytokines (TNF-α, IL-1,6) activate inducible nitric oxide synthetase (iNOS) to produce NO. NO is metabolized to nitrite, which has a short half-life in blood, and then to nitrate. Estimation of NO activity can be made by measuring nitrites, nitrates or both. It has been hypothesized that NO levels may be elevated in severe leptospirosis. In fact, NO levels have been shown to be significantly elevated in patients with symptomatic leptospirosis [13,14]. Similar patterns have been demonstrated in malaria. Paradoxically however, in malaria, when NO levels were corrected for renal function, it was demonstrated that total NOx (nitrite + nitrate) levels were actually lower in patients with severe malaria [15]. In severe leptospirosis, since there is often renal impairment, it is possible that the raised NO levels reflect reduced renal clearance of NO rather than increased synthesis. Kalugalage et al. demonstrated that, similar to what was seen in malaria, the corrected total NOx (nitrite+nitrate) concentration (i.e., corrected for renal impairment) in patients with severe leptospirosis was actually lower than in patients with mild leptospirosis and non-leptospirosis fever [16]. The pathophysiological basis for this pheno-menon remains elusive. Whether a blunted iNOS response contributes to the development of severe disease or whether low levels are a result of severe disease is unclear. Furthermore, there is limited evidence as to which meta-bolite/s, i.e., nitrite, nitrate, or both together, most accu-rately reflects NO activity in response to severe infection. Nitrite is likely to be more specific, as it has a short half life, and is less affected by renal function.

There has been considerable interest in the role of oxidative stress in the pathogenesis of severe disease, including leptospirosis. NO is one of the mediators which drives oxidative stress, and, like in many other diseases, oxidative stress is likely to play a role in tissue and organ damage in leptospirosis; current evidence on this is limited. It is possible that high levels of NO fuel increased oxidative stress, and that this is one of the pathways through which tissue and organ damage occurs in severe leptospirosis.

Why do some individuals develop severe disease while others develop a mild febrile illness? There is little evidence as yet whether infection with certain pathogenic serovars, or whether bacterial load contributes to severity of infection. Whether genetic variations in the host

immune response predispose certain individuals to severe leptospirosis is also largely unknown.

Proteomics is a potentially useful tool to study the variations in the inflammatory response which occurs in acute leptospirosis in different individuals. Differential expression of specific proteins in patients with leptospirosis has been demonstrated previously [17]. Genetic polymorphisms of cytokine genes may also influence the host response to leptospirosis; for example certain HLA alleles, and also polymorphisms of interleukin genes have been shown to be associated with leptospirosis. This is an area with great potential for further study. Such prognosticomics clusters may help prognostication of leptospirosis, and this is an evolving field [18].

Control measures for leptospirosis will help reduce the burden of the disease. Nonetheless there is a great need for clinically relevant basic sciences research in leptospirosis. Unraveling the complex mechanisms which lead to the pathogenesis of severe disease will lead to the identification of biomarkers which could predict severity, guiding clinicians to allocate and institute intensive care in a timely manner, and transfer patients to hospitals with better facilities early. Furthermore, studies in this area could potentially lead to interventions which could halt the progression to severe multi-organ failure in leptospirosis.

References

1. Levett PN. Leptospirosis. *Clinical Microbiology Reviews* 2001; **14**: 296-326.
2. Sarkar J, Chopra A, Katageri B, *et al*. Leptospirosis: a re-emerging infection. *Asia-Pacific Journal of Tropical Medicine* 2012; **5**: 500-2.
3. Smith JK, Young MM, Wilson KL, Craig SB. Leptospirosis following a major flood in Central Queensland, Australia. *Epidemiology and Infection* 2012; **25**: 1-6.
4. Agampodi SB, Karunarathna D, Jayathilala N, *et al*. Outbreak of leptospirosis after white-water rafting: sign of a shift from rural to recreational leptospirosis in Sri Lanka? *Epidemiology and Infection* 2014; **142**: 843-6.
5. Rodrigo C, Lakshitha de Silva N, Goonaratne R, *et al*. High dose corticosteroids in severe leptospirosis: a systematic review. Transactions of the Royal Society of Tropical Medicine and Hygiene 2014 (epub ahead of print).
6. Limmathurotsakul D, Turner EL, Wuthiekanun V, *et al*. Fool's gold: Why imperfect reference tests are undermining the evaluation of novel diagnostics: a reevaluation of 5 diagnostic tests for leptospirosis. *Clinical Infectious Diseases* 2012; **55**: 322-31.
7. De Silva NL, Niloofa M, Fernando N, *et al*. Changes in full blood count parameters in leptospirosis: a prospective study. *International Archives of Medicine* 2014; **7**: 31.
8. Rajapakse S, Rodrigo C, Hannifa R. Predictors of mortality in severe leptospirosis; a concept paper on developing a clinically relevant classification. *Journal of Emergencies, Trauma and Shock* 2010; **3**: 213-9.
9. De Fost M, Chierakul W, Limpaiboon R, *et al*. Release of granzymes and chemokines in Thai patients with leptospirosis. *Clinical Microbiology and Infection* 2007; **13**: 433-6.
10. Wagenaar JF, Gasem MH, Goris MG, *et al*. Soluble ST2 levels are associated with bleeding in patients with severe Leptospirosis. *PLoS Neglected Tropical Diseases* 2009; **3**: e453.
11. Wagenaar JF, Goris MG, Gasem MH, *et al*. Long pentraxin PTX3 is associated with mortality and disease severity in severe Leptospirosis. *Journal of Infection* 2009; **58**: 425-32.
12. Limper M, Goeijenbier M, Wagenaar JF, *et al*. Copeptin as a predictor of disease severity and survival in leptospirosis. *Journal of Infection* 2010; **61**: 92-4.
13. Maciel EA, Athanazio DA, Reis EA, *et al*. High serum nitric oxide levels in patients with severe leptospirosis. *Acta Tropica* 2006; **100**: 256-60.
14. Gunaratna RI, Handunnetti SM, Bulathsinghalage MR, *et al*. Serum nitrite levels in Sri Lankan patients with leptospirosis. *Asia-Pacific Journal of Tropical Medicine* 2012; **5**: 75-8.
15. Anstey NM, Granger DL, Hassanali MY, *et al*. Nitric oxide, malaria, and anemia: inverse relationship between nitric oxide production and hemoglobin concentration in asymptomatic, malaria-exposed children. *American Journal of Tropical Medicine and Hygiene* 1999; **61**: 249-52.
16. Kalugalage T, Rodrigo C, Vithanage T, *et al*. Low serum total nitrite and nitrate levels in severe leptospirosis. *BMC Infectious Diseases* 2013; **13**: 206.
17. Srivastava R, Ray S, Vaibhav V, *et al*. Serum profiling of leptospirosis patients to investigate proteomic alterations. *Journal of Proteomics* 2012; **76**: 56-68.
18. Jayasinghe S. 'Prognostic -Omic Clusters' (POCs): a novel approach to health and disease. *Medical Hypotheses* 2014; **82**: 703-5.

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