Early identification of leptospirosis-associated pulmonary hemorrhage syndrome by use of a validated prediction model

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Summary   *Objective:* To identify prediction factors for the development of leptospirosis-associated pulmonary hemorrhage syndrome (LPHS).
Methods: We conducted a prospective cohort study. The study comprised of 203 patients, aged ≥14 years, admitted with complications of the severe form of leptospirosis at the Emílio Ribas Institute of Infectology (Sao Paulo, Brazil) between 1998 and 2004. Laboratory and demographic data were obtained and the severity of illness score and involvement of the lungs and others organs were determined. Logistic regression was performed to identify independent predictors of LPHS. A prospective validation cohort of 97 subjects with severe form of leptospirosis admitted at the same hospital between 2004 and 2006 was used to independently evaluate the predictive value of the model.
Results: The overall mortality rate was 7.9%. Multivariate logistic regression revealed that five factors were independently associated with the development of LPHS: serum potassium (mmol/L) (OR = 2.6; 95% CI = 1.1–5.9); serum creatinine (μmol/L) (OR = 1.2; 95% CI = 1.1–1.4); respiratory rate (breaths/min) (OR = 1.1; 95% CI = 1.1–1.2); presenting shock (OR = 69.9; 95% CI = 20.1–236.4), and Glasgow Coma Scale Score (GCS) < 15 (OR = 7.7; 95% CI = 1.3–23.0). We used these findings to calculate the risk of LPHS by the use of

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a spreadsheet. In the validation cohort, the equation classified correctly 92% of patients (Kappa statistic = 0.80).

Conclusions: We developed and validated a multivariate model for predicting LPHS. This tool should prove useful in identifying LPHS patients, allowing earlier management and thereby reducing mortality.

Introduction

Leptospirosis, a spirochetal zoonosis, has been increasingly recognized as an important cause of pulmonary hemorrhage syndrome.[1—4] Five to fifteen percent of the clinical infections progresses to develop severe disease manifestations.[1—3,5] The Nicaragua outbreak in 1995 raised awareness of leptospirosis as the cause of severe pulmonary hemorrhage.[4] Whereas case fatality is 5—15% for Weil's disease, it is more than 50% in patients who develop leptospirosis-associated pulmonary hemorrhage syndrome (LPHS).[5—8]

Early identification and triage of patients at risk for developing LPHS is essential to reduce the high case fatality rate. LPHS patients require intensive care unit (ICU) monitoring and aggressive supportive care for concomitant acute respiratory distress syndrome (ARDS), acute kidney injury and hypotension.[9—14] Protective mechanical ventilation modalities[14] and daily hemodialysis[15] have been shown to provide beneficial outcomes in clinical trials which included leptospirosis patients as subjects. However, identification of patients at risk for developing LPHS is difficult,[10—14] especially during initial hospital evaluation. Therefore predictive markers (e.g. hematology-test and biochemistry-test) need to be identified such that patients at risk for developing LPHS can be effectively identified and triaged.

We herein report the findings of a study which aimed to develop and validate a predictive model which can be used to identify patients at risk for developing LPHS at the time of hospital admission.

Patients and methods

Patient cohorts

A prospective cohort study was performed at Emílio Ribas Institute of Infectology, the state infectious disease hospital in Sao Paulo, Brazil. This 200-bed hospital serves as the reference center for leptospirosis in the city which has a population of 10 million inhabitants. From October 1998 through December 2006, the study team of clinicians reviewed hospital admission records to consecutively identify suspected cases of leptospirosis during the first 24 h of hospitalization. Patients were prospectively enrolled into the cohort who had suspected leptospirosis based on clinical and laboratory criteria established by the Brazilian Ministry of Health,[16] were hospitalized at the study site, had age greater or equal to 14 years and provided written informed consent to participate in the study, according to protocols approved by Committee of Emílio Ribas Institute of Infectology and the National Ethics Committee in Research for the Brazilian Ministry of Health. Patients were excluded from the study if they had laboratory or radiologic evidence for a disease other than leptospirosis during hospitalization and did not have laboratory-confirmed diagnosis of leptospirosis. Other diseases causing bleeding were ruled out by serological testing and blood cultures.

Data collection

Information was obtained on potential predictive factors for LPHS at the time of initial emergency room evaluation. A standardized data form was used to extract information on demographic characteristics, clinical signs and symptoms and laboratory findings from the medical records. A complete history, physical examination, complete blood count, serum electrolytes and coagulation parameters (prothrombin and prothromboplastin time) were obtained as part of routine emergency room evaluation for suspected leptospirosis. Haemoptysis was encoded as expectoration of blood-tinged sputum. The Glasgow Coma Scale (GCS) was used to assess the mental status of subjects. Arterial blood gas evaluation was performed on a subset of patients who had signs of dyspnea and hypotension. Diuresis was assessed by evaluating urinary volume during the first 24 h of hospital admission. Oliguria was defined as a 24 h urinary volume of less than 400 mL. Shock was defined as the use of vasoactive drugs (one or more) to maintain a systolic blood pressure >90 mmHg after fluid resuscitation in the first 24 h of hospitalization. The study team determined the Acute Physiology and Chronic Health Evaluation (APACHE) II score for patients based on findings obtained during emergency room evaluation.

During hospitalization all patients received antimicrobial therapy with penicillin, ampicillin or ceftriaxone. When indicated, patients received intravenous fluid resuscitation, vasoactive drugs, supplemental oxygen, blood component transfusions and ICU monitoring. Intravenous fluid overload was avoided in patients with ARDS and pulmonary edema. Renal replacement therapy was initiated for indications of oliguria, hyperkalemia, severe acidosis, fluid overload and uremic syndrome. The study team followed patients daily during hospitalization to evaluate outcomes of LPHS, defined as massive pulmonary bleeding (hemoptysis > 300 ml or aspiration of fresh blood after endotracheal intubation which did not clear with suctioning) and respiratory failure requiring mechanical ventilation, and mortality.

Laboratory confirmation

Serum samples were collected at the time of hospital admission and a period of 10—14 days afterwards during convalescence. The IgM ELISA assay, microscopic agglutination test (MAT) and culture isolation were performed as previously reported. Immunohistochemical and immunofluorescence studies of autopsy specimens were routinely performed with anti-*Leptospira* antibodies.[1,3,7,8,10] A laboratory-

confirmed diagnosis of leptospirosis was defined as a positive IgM ELISA result, seroconversion or four-fold rise in the MAT titer between acute- and convalescent-phase samples, a single MAT titre $\geq$ 1:800, a positive *Leptospira* culture or identification of leptospires in autopsy specimens, detected through culture or immunohistochemical staining, in clinical specimens. The presumptive infecting serogroup was determined based on the serovar against which highest agglutination titres were directed.

Statistical analysis

The prospective cohort was divided into two groups, a derivation cohort that was enrolled from January 1998 through October 2004 and a validation cohort that was enrolled from November 2004 through December 2006. Bivariate analyses were performed with information from the derivation cohort to identify clinical and laboratory findings at the time of hospital admission which were associated with the risk of developing LPHS during hospitalization was compared to those of patients who did not develop LPHS. Continuous variables were evaluated for their distribution. The Mann–Whitney and chi-square test was used to compare continuous and categorical variables, respectively, for patients who did and did not develop LPHS. Significant differences were defined as a *P*-value <0.05. Odds ratios (OR) and 95% confidence intervals (95% CI) were calculated to estimate the risk of developing LPHS associated with baseline findings.

Logistic regression was performed to identify independent predictors of LPHS. Variables which were found to be significant in the bivariate analyses were included in the regression analysis. Continuous variables were maintained as such in the model, unless preliminary analyses indicated that categorization of the variable improved the predictive value. Due to co-variation among clinical and laboratory findings, variables were grouped into categories according to pulmonary, renal, hepatic and hemodynamic complications. A forward stepwise approach was used to identify significant independent predictors of LPHS within each group. To develop a prediction rule, a scoring system was developed based on multiplying by different values the natural logarithm of the OR for each risk factor identified in the logistic regression analysis and rounding the value to the nearest integer. However we did not identify a point scoring system that had sufficient predictive value in comparison to the regression model. Therefore the predictive model used in the study was based on the exponential equation derived from the logistic regression analysis.

Cutoff points were determined by constructing a receiver operating characteristic (ROC) curves. Based on the evaluation of a range of threshold criteria for the predictive model, a final criteria of Pr(LPHS) of $\geq$0.5 was used. An Excel spreadsheet to calculate risk of LPHS is available as supplementary material.

Information from the validation cohort was used to independently evaluate the predictive value of the model. The discriminatory ability of the predictive model was used to assess by calculating the area under the ROC curve. Sensitivity, specificity and positive and negative predictive values and their 95% CI for the predictive model were calculated for the derivation, validation and combined cohorts, using standard

definitions and methods. All analyses were performed using STATA version 8.0 (Stata Corporation, College Station, TX, USA).

Results

Derivation cohort

From January 1998 through October 2004, 281 patients with suspected leptospirosis were hospitalized at the study site. Of these 27 patients were excluded because they had evidence of disease other than leptospirosis or did not have a laboratory-confirmed diagnosis of leptospirosis and 51 were excluded for being under 14 years of age. Of the 203 subjects enrolled in the prospective derivation cohort, 172, 29 and 2 had a confirmed diagnosis on the basis of MAT, IgM ELISA and culture isolation criteria, respectively. The presumptive infecting serogroup was *Leptospira interrogans* serogroup Icterohaemorrhagiae in 71% (144) of the patients.

The most common infecting serovars (%) in the LPHS group was *L. interrogans* serovar Icterohaemorrhagiae (38%), followed by *L. interrogans* serovar Copenhageni (33.3%), *Leptospira kirschneri* serovar Cynopteri (23.8%), and *L. interrogans* serovar Canicola (4.8%), and *L. kirschneri* serovar Grippotyphosa (4.7%). Among the non-LPHS group infecting serovars was *L. interrogans* serovar Icterohaemorrhagiae (32%), followed by *L. interrogans* serovar Copenhageni (31%), *L. kirschneri* serovar Grippotyphosa (7.8%), *L. kirschneri* serovar Cynopteri (7.8%), *L. interrogans* serovar Canicola (4.4%), and *L. interrogans* serovar hebdomadis (1.1%), *L. interrogans* serovar Pomona (1.1%), and *L. interrogans* serovar Pyogenes (1.1%).

Subjects in the derivation cohort had clinical manifestations of Weil's disease. Acute kidney injury, defined as a serum creatinine level $\geq$176.8 μmol/L, oliguria and jaundice were observed in 70.9% (144), 10% (21) and 86.7% (176), respectively, of the patients, while 26.1% (53) had signs of gastrointestinal bleeding, hemoptysis or others hemorrhagic manifestations. Of note, significant differences were observed between LPHS and non-LPHS patients with respect to hemorrhagic manifestations at hospital admission 39% *vs.* 22%, respectively, *P* = 0.01. No statistically difference was seen in LPHS and non-LHPS in chest x-rays at hospital admission 23.5% *vs.* 17.5%, respectively, *P* = 0.41. During the first 24 h of hospitalization, 21% (43) developed shock.

During hospitalization, 132 (65%) and 28% of the patients required ICU monitoring and dialysis. Among the 203 patients, 51 (25%) developed LPHS based on the findings of massive pulmonary hemorrhage and respiratory failure requiring mechanical ventilation. The overall case fatality for the derivation cohort was 8% (16 deaths). All of the deaths occurred among patients who had developed LPHS (16 of 51 patients; case fatality rate, 31%).

Risk factors for developing LPHS during hospitalization

Table 1 shows the clinical and laboratory findings at the time of hospital admission for patients who did and did not develop LPHS. Of the 203 subjects, 167 (82.3%) presented serologic

Table 1 Age, clinical signs and laboratory values obtained at admission in 203 patients with severe leptospirosis.

Factor	Normal values	LPHS				P value
		Absent		Present		
		n	Mean (SD)	n	Mean (SD)	
Demographic						
Age (years)		152	33.5(1.0)	51	39.3(2.0)	0.015
Sex (male/female) (*n*/%)		152	142/10	51	46/5	0.44
Renal involvement						
Blood urea nitrogen (mmol/L)	2.1–7.1	152	41.0(2.35)	51	54.8(3.8)	<0.001
Creatinine (μmol/L)	70–120	152	309.4(17.7)	51	433.2(35.4)	<0.001
Potassium (mmol/L)	3.5–4.5	152	3.6(0.04)	51	3.9(0.08)	0.014
Hepatic involvement						
Aspartate aminotransferase (μkat/L)	0–0.58	151	1.97(0.15)	51	3.2(0.45)	0.002
Total bilirubin (μmol/L)	2–21	149	217.2(15.4)	51	307.8(29.1)	0.002
Albumin (g/L)	34–47	100	28(0.4)	43	24(0.6)	<0.001
Muscle involvement						
Creatine kinase (μkat/L)	0.53–4.45	124	15.8(29.2)	45	38.5(41.2)	<0.001
Hematologic involvement						
WBC count ($\times 10^9$/L)	4.8–10.8	152	11.0(4.8)	51	13.7(7.7)	0.02
Haematocrit	39–49	151	37.0(5.9)	51	33.8(6.4)	0.001
Platelet count ($\times 10^9$/L)	150–450	152	103.0(7.7)	51	63.0(4.9)	<0.001
Prothrombin time (sec)	11–15	120	14.0(5.0)	47	13.2(2.4)	0.47
Respiratory manifestations						
Respiratory rate (n/min)	12–20	152	23.6(7.5)	51	31.4(11.8)	<0.001
Arterial oxygen (kPa)	11.04–14.36	118	10.9(3.3)	51	10.1(4.1)	0.18
PaO_2/FiO_2 (ratio)	>350	120	383.7(76.9)	51	290.4(123.4)	<0.001
Arterial pH (units)	7.35–7.45	118	7.42(0.05)	51	7.34(0.1)	<0.001
Arterial bicarbonate (mmol/L)	22–29	118	18.1(3.8)	51	16.0(4.3)	0.002
Cardiac manifestations						
Heart rate (*n*/min)	60–100	152	90.2(19.0)	51	106.4(16.6)	<0.001
MAP (mmHg)	80–90	152	88.8(16.1)	51	84.2(21.5)	0.10
Monitoring						
Fever (*n*/%)	No	152	123(80.9)	51	37(74)	0.29
Pulse oximetry (SaO_2)	0.95–1.0	118	0.94(0.4)	51	0.88(0.1)	<0.001
APACHE II (score)	0	146	9.0(4.5)	51	15.4(4.9)	<0.001
Shock	No	162	145	41	34	<0.001
GCS < 15	15	178	143	23	15	<0.001
Diuresis (mL/24 h)	800–2000	121	2732(168)	44	1816(293)	0.001

LPHS = leptospirosis-associated pulmonary hemorrhage syndrome; WBC = white blood cells; PaO_2 = arterial oxygen tension; FiO_2 = fraction of inspired oxygen; MAP = mean arterial pressure; SaO_2 = arterial oxygen saturation; APACHE = acute physiological and chronic health evaluation; shock = hemodynamic instability in the first 24 h; GCS = Glasgow Coma Scale.

evidence of leptospirosis on the MAT and 71% (100) infected with the serogroup Icterohaemorrhagiae. Significant differences were not found between the two groups with respect to symptoms of fever, headache, myalgia or gastrointestinal manifestations (results not shown), and between the infecting serogroup in terms of organ involvement, pulmonary hemorrhage or death. LPHS patients had significantly increased frequency of having renal, hepatic, respiratory and cardiovascular complications and altered mental status at the time of hospital admission (Table 1).

Logistic regression analysis identified five independent risk factors for developing LPHS based on clinical and laboratory parameters at the time of admission (Table 2).

The presence of shock (OR = 41.4; 95% CI = 20.1–236.4) and GCS < 15 (OR = 5.5; 95% CI = 1.3–23.0) was the strongest predictor of LPHS. The model correctly classified 88% of the subjects in the derivation cohort, with a sensitivity of 73% and a specificity of 93%. A point scoring system was not identified which had sufficient performance as compared with the predictive model.

The prediction equation for LPHS is as follows:

Pr(LPHS) = exp {−11.0 + 0.12 × respiration rate + 0.23 × serum creatinine + 4.39 (if shock present) + 0.98 × serum potassium + 2.09 (if GCS score < 15)}/1+ exp {−11.0 + 0.12 ×respiration rate + 0.23 × serum creatinine + 4.39

Table 2 Crude and adjusted odds ratios and 95% confidence intervals for risk factors associated with leptospirosis-associated pulmonary hemorrhage syndrome.

Factor	Crude OR	Adjusted OR[b] (95% CI)	P value
Potassium (mmol/L)[a]	1.9	2.6 (1.1—5.9)	0.02
Serum creatinine (μmol/L)[a]	1.2	1.2 (1.1—1.4)	0.003
Respiratory rate (n/min)[a]	1.1	1.1 (1.1—1.2)	<0.0001
Shock			
No	1.0[c]	—	
Yes	41.4	69.2 (20.1—236.4)	<0.0001
Glasgow Coma Scale			
15	1.0[c]	—	
<15	7.7	5.5 (1.3—23.0)	0.02

Shock = hemodynamic instability in the first 24 h.
[a] Used as continuous variables in the prediction model.
[b] Adjusted for the other factors shown in the table.
[c] Reference group.

(if shock present) $+ 0.98 \times$ serum potassium $+ 2.09$ (if GCS score < 15)}

Validation cohort

A second independent and prospective cohort of 97 patients who fulfilled the study criteria was enrolled from November 2004 to December 2006. Acute kidney injury, defined as a serum creatinine ≥ 176.8 μmol/L, oliguria, jaundice and shock were observed in 72.6% (70), 6% (6), 70.1% (67) and 49% (48), respectively, of the patients. During hospitalization, 29 (29.9%) developed LPHS. The overall case fatality for the validation cohort was 6.8% (6 deaths). All of the deaths occurred among patients who had developed LPHS.

Accuracy of prediction model in identifying patients who developed LPHS

We assessed the performance of the logistic regression model in predicting pulmonary hemorrhage by constructing the ROC curve (Fig. 1). The area under the curve was 0.94 (sensitivity: 72.5%; specificity: 93.4%; positive predictive value: 78.7%; negative predictive value: 91%).

We compared the performance of the predictive model in identifying LPHS during hospitalization with the APACHE II scoring system. The area under the ROC curve was significantly larger for the predictive model than the APACHE II scoring system (0.94; 95% CI = 0.91—0.98 *vs.* 0.83; 95% CI = 0.76—0.90; $P < 0.05$). The sensitivity, specificity, positive predictive value and negative predictive values for the APACHE II scoring system were 57%, 91%, 67% and 86%, respectively, and were overall lower than those for the predictive model.

The equation was applied to the validation cohort, and 89 (92%) patients were correctly classified. In the non-LPHS group, 6 cases were judged to have LPHS, whereas 2 cases in the LPHS group were judged to not have LPHS (Kappa statistic = 0.80).

Area under ROC curve = 0.9444
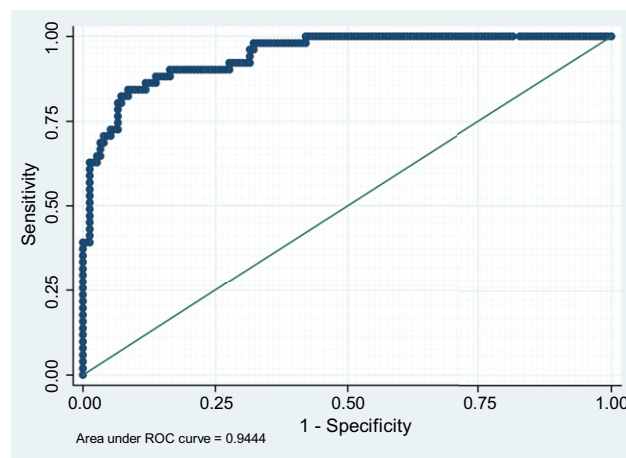
Discussion

Leptospirosis presents a wide variety of clinical manifestations and prognoses. Leptospirosis presenting as LPHS is associated with a high mortality rate. However, the clinical course of LPHS can be improved by early diagnosis and prompt treatment.

In the present study, we developed and validated a multivariate prediction model for LPHS based on five readily available variables. Assigning patient values to each variable, the equation we developed can be used to estimate individual risk. This prediction model proved to be excellent tool for distinguishing between patients with LPHS and those with other severe forms of leptospirosis (area under the ROC curve = 0.94). This constitutes a new tool that, using data routinely available at hospital admission, provides physicians with a simple method of accurately assessing LPHS risk. Patients in the high-risk group would benefit the most from rapid triage to a reference center in order to receive aggressive therapies, including monitoring, hemodynamic resuscitation, invasive mechanical ventilation and early dialysis.

To our knowledge, this is the first study using multivariate analysis to assess clinical factors associated with LPHS and proposing a tool to predict patient risk at hospital admission. However, previous studies included similar factors in prognostic models for leptospirosis. Panaphut et al.[5] found that oliguria, hyperkalemia, pulmonary rates or hypotension on admission in patients with leptospirosis were predictors for mortality. Niwattayakul et al. and Doudier et al.[9,17] found that hypotension, oliguria and abnormal chest auscultation were related to prognosis. As with our results, Esen et al.[18] demonstrated that altered mental status and potassium levels at the time of hospital admission were seen to be independent risk factors for motality.[5,7,8,18] The higher serum potassium levels may have been provoked by more severe renal dysfunction, metabolic derangement, or rhabdomyolisis.[8,18] Altered mental status has previously been reported as a powerful independent risk factor for mortality.[7,18] The laboratory findings in LPHS patients in the present study are consistent with the observations made by Craig et al.[19] When leptospirosis is suspected, patients presenting with

higher levels of blood urea, serum creatinine, and leukocytosis and lowers levels of serum albumin, haematocrit and platelets may need to be closely monitored and have aggressive treatment regimes directed toward them.[19]

It is no coincidence that our findings and those of such previous studies point to similar factors, LPHS being the main cause of death among patients with leptospirosis.[6–9,11–15]

Another strength of our study is the higher number of consecutive patients included in our cohort — more than 200 patients, 25% of them with LPHS. In addition, all of them had been admitted to the same hospital, which resulted in homogenous criteria at admission and during follow-up evaluations. Finally, our model was validated in an independent sample of 97 consecutive patients, 29% of them with LPHS, hospitalized with severe leptospirosis, in which the use of our equation resulted in the correct classification of 92% of the patients.

Certain limitations of this model should be considered. The model includes only variables available from our data set, although there could be other factors affecting the outcome which are not routinely collected in our center. In addition, although the data used to develop the model were subjected to local validation, the performance of the model in predicting LPHS should be tested in primary care hospitals and in areas of lower incidence. Furthermore, the validation in another settings would be desirable.

In conclusion, we have developed a multivariate prediction model, based on three clinical factors and two laboratory testing factors, to predict LPHS risk in an urban area with a high incidence of leptospirosis. These variables are, in descending order of statistical significance, as follows: hemodynamic instability (shock within the first 24 h), altered mental status (GCS score < 15), serum potassium, serum creatinine and respiratory rate. The model can be used at hospital admission to calculate patient-specific risk based on the logistic equation. This tool could also be useful to clinicians in the identification of patients with LPHS and should help to reduce mortality by allowing earlier management of these cases.

Conflict of interest

The authors state that there is no conflict of interest.

Acknowledgements

Appendix. Supplementary data

Supplementary data associated with this article can be found, in the on-line version, at doi:10.1016/j.jinf.2009.12.005.

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