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Acute Respiratory Distress Syndrome Secondary to Influenza A(H1N1)pdm09: Clinical Characteristics and Mortality Predictors

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ABSTRACT

Background: Acute respiratory distress syndrome secondary to influenza A(H1N1)pdm09 virus is the leading cause of death among this patient population. Expanding the knowledge of its course and predictors of mortality is relevant to decision making. We aimed to describe the clinical characteristics and identify factors associated with mortality in patients with acute respiratory distress syndrome secondary to influenza A(H1N1)pdm09 during the 2013-2014 influenza season. **Methods:** This is an observational study of a prospective cohort of 70 patients with acute respiratory distress syndrome and influenza A(H1N1) pdm09 seen in an academic medical center. Multivariate logistic regression was used to identify the independent mortality predictors. Bootstrap was used for internal model validation. **Results:** This cohort was represented by young adults (43 ± 11 years old). Obesity was present in 62.5% and was not associated with mortality. Mortality at 28 days and at discharge from the respiratory intensive care unit was 14 and 20%, respectively. All patients met the criteria for acute respiratory distress syndrome, 73% had vasodilatory shock, and 27.1% had acute kidney injury on respiratory intensive care unit admission. We observed a high incidence of intensive care unit-acquired weakness (81.4%). Ventilator-associated pneumonia developed in 47.1% and was not associated with mortality. In multivariate analysis, independent risk factors for intensive care unit mortality were age (odds ratio [OR] = 1.102), white blood cell count (OR = 1.22), and lactate dehydrogenase levels (OR = 1.004) on admission to the intensive care unit. **Conclusions:** We described the clinical characteristics and course of a cohort of patients with acute respiratory distress syndrome secondary to influenza A(H1N1)pdm09, and developed a predictive model of mortality based on the covariates age, levels of lactate dehydrogenase, and white cell count on admission to the respiratory intensive care unit. (REV INVES CLIN. 2016;68:235-44)

Key words: ARDS. Influenza A(H1N1)pdm09. Mortality. Prognostic factors. 2013-2014 influenza season.

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INTRODUCTION

In March 2009, the first cases of a novel respiratory infection caused by influenza virus A(H1N1)pdm09 were reported in Mexico. In contrast to the seasonal influenza virus, infection with the A(H1N1)pdm09 virus had a higher prevalence in young adults and obese and pregnant women and was associated with severe pneumonia, requiring frequent admission to the intensive care unit for mechanical ventilation and multisystem organ support. High mortality was reported in Mexico among patients who had acute respiratory failure and required mechanical ventilation[1,2]. This mortality contrasted strongly with that reported by other groups during the 2009 influenza pandemic[3,4].

Following the 2009 influenza pandemic, new outbreaks occurred in 2010-2011[5] and most recently from October 2013 through May 2014. During this last influenza season, a substantial increase was documented in the number of A(H1N1)pdm09-related hospitalizations and deaths, as was a proportionate shift of severe disease affecting middle-aged adults compared with the preceding A(H1N1)pdm09 2011-2012 epidemic in Mexico[6]. However, there are no reports on demographics, clinical characteristics, and outcome of those patients who developed severe pneumonitis and acute respiratory distress syndrome (ARDS) during the 2013-2014 influenza A(H1N1) pdm09 season. Increased knowledge on its course and predictors of mortality is relevant to decision making.

The objectives of this study were to describe the clinical characteristics and identify factors associated with mortality in patients admitted to our respiratory intensive care unit (RICU) presenting with ARDS secondary to viral pneumonitis by A(H1N1)pdm09 virus during the 2013-2014 influenza season.

MATERIAL AND METHODS

Study design and patient population

This is an observational study of a prospective cohort of patients admitted to the RICU from October 2013 through May 2014. We included all patients admitted to the RICU requiring mechanical ventilation and multisystem support for acute respiratory failure caused by primary viral pneumonia due to influenza A(H1N1) pdm09.

Nasopharyngeal swab specimens were collected on admission; lower respiratory secretions were also obtained in patients who had been intubated. Real-time polymerase chain reaction testing (RT-PCR)[7] was performed for A(H1N1)pdm09 and additional samples were obtained for testing with a multiplex PCR assay for coinfection with other respiratory pathogens (Allplex™, Respiratory Full Panel Assay, Seegene Technologies, Inc., USA.). A confirmed case was defined as a patient with an acute respiratory illness with laboratory-confirmed pandemic A(H1N1)pdm09 by strain-specific PCR. The study was approved by the institutional ethics committee, and the requirement of informed consent was waived due to the observational nature of the study.

Data collection and definition of variables and outcome

Data were obtained by two investigators and stored in an electronic database. The following variables were recorded: demographics, immunization status, comorbidities, time elapsed from the onset of symptoms to hospital admission, time to the first dose of oseltamivir, signs and symptoms, previous use of antibiotics, microbiological findings, and chest radiological findings. Cardiovascular and respiratory variables, parameters of mechanical ventilation, laboratory studies, and organ failure scores were evaluated on admission to the RICU. Organ failure was assessed using the Sequential Organ Failure Assessment (SOFA) scoring system[8]. Patients were followed throughout the course of their illness and development of complications was recorded.

Acute respiratory distress syndrome was defined according to the recent Berlin Definition[9]. Primary viral pneumonia was defined as the presence of pulmonary infiltrates in two or more lobules with negative cultures for bacteria in respiratory secretions and blood during the influenza season. Coinfection was defined as microbial growth in cultures of tracheal aspirates or bronchoalveolar lavage obtained within 48 hours of hospital admission. Ventilator-associated bacterial pneumonia was considered when there was recurrence of fever, leukocytosis, purulent sputum, and new infiltrates in a setting of greater respiratory impairment, plus positive cultures of respiratory secretions[10]. Acute kidney

injury (AKI) was defined according to the classification reported by Metha, et al.[11]. Oseltamivir was administered by a nasogastric tube at doses of 150-300 mg/24 hours as recommended by the World Health Organization (WHO)[12], with doses selected by the attending physician. Criteria for ICU admission and treatment decisions were not standardized for all patients and were decided by the attending physician. Outcomes were defined as 30- and 90-day all-cause mortality.

Statistical analysis

Data were described as counts or percentages for categorical variables, and as means and standard deviations (SD) or medians and interquartile ranges (IQR) for continuous variables. Differences between characteristics of the groups were analyzed using Student's *t*-test for independent samples or the Mann-Whitney rank-sum test for continuous variables, and the chi-square test or Fisher's exact test for categorical variables. Multivariate logistic regression was used to evaluate the association between clinical characteristics and mortality. All the factors associated with mortality in the univariate analysis were entered into the multivariate analysis with backward elimination ($p < 0.05$ to retain) to select the final set of predictors. The same model was selected using a forward stepwise procedure ($p < 0.05$ for admission). Results are presented as odds ratio (OR) and 95% confidence intervals (CI). Internal validation of the model was obtained by the statistical bootstrap procedure. In total, 1000 random samples with replacement were obtained from the original cohort. The model was developed again in each bootstrap sample yielding the new parameters' values to each bootstrap model. Finally, the mean, standard error, and confidence intervals of coefficients were obtained. Odd ratios were calculated as the exponential of coefficients. The distribution of the times of survival was analyzed by using the Kaplan-Meier method. A log rank test was run to determine if there were differences in the survival distribution for relevant covariates. The resulting model's predictive value was evaluated using a receiver operating characteristic (ROC) area under the curve (AUC). Confidence intervals and p values were considered significant for a two-tailed alpha level of 0.05. Data analysis was performed using SPSS for Windows 21.0 (SPSS, Inc, Chicago, IL, USA).

RESULTS

Characteristics of the study patients

Tables 1 and 2 show the demographic and clinical characteristics, comorbidities, physiological responses, and outcomes of patients. The average age was 47 ± 11 years; 61.4% were men and only 5% had been immunized against influenza. The median time to seek medical attention was three (IQR: 2-4) days, the median time from the onset of symptoms to admission to the emergency department was eight (IQR: 7-10) days, the median time to receive the first dose of oseltamivir was eight (IQR: 7-10) days, and 95.2% of patients received one or more antibiotics before admission. Obesity was the most common comorbidity, present in 62.5% of patients. The main symptoms were cough (100%), sore throat (59.4%), subjective feeling of fever (98.5%), shortness of breath (100%), headache (67.7%), intense fatigue (100%), and myalgia (84.6%). Hemoptysis was present in 36.9% of patients. On physical examination, the most common findings were crackles (96.9%), wheezing (15.4%), and cyanosis (52.1%).

Physiological response and severity of illness in the respiratory intensive care unit at admission

All patients required invasive mechanical ventilation. The partial pressure of arterial O_2 to the fraction of inspired O_2 (PaO_2/FIO_2) ratio was 95 ± 39, the median time on mechanical ventilation was 17 (IQR: 8-29) days, and 72.8% of patients required vasopressors. The median lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) levels on admission were 590 (IQR: 368-867) U/l and 764 (IQR: 226-1,894) U/l, respectively. The median SOFA score was 8 (IQR: 5-9). The median length of stay at the RICU was 18 (IQR: 1-32) days. Mortality at 28 days was 14.2% and at discharge from the RICU, 20%.

Comparison between survivors and non-survivors

Demographic data, clinical characteristics and comorbidities of patients who survived compared to those who did not survive are shown in table 2. Non-survivors were older than survivors (49 ±11 vs. 42 ± 10 years;

Table 1. Demographics, comorbidities, and clinical data of survivors and non-survivors

Patient characteristics on admission	All patients (n = 70)	Survivors (n = 56)	Non-survivors (n = 14)	*p* value
Age (years)	43 ± 11	42 ± 10	49 ± 11	0.02
Gender (M%)	61.4	61.4	61.5	0.80
BMI (kg/m^2)	31 ± 6.8	32.2 ± 6.9	30.7 ± 6.4	0.62
Influenza immunization (%)	5.7	7.0	0	0.57
Duration of symptoms to primary medical care, median (IQR) days	3 (2-4)	3 (2-4)	2 (1-3)	0.12
Previous medical care (%)	97.7	100	92.3	0.20
Duration of symptoms to hospital admission, median (IQR) days	8 (7-10)	8 (7-10)	7 (5-8)	0.35
Interval from symptom onset to swab, median (IQR) days	8 (7-10)	8 (7-10)	7 (5-8)	0.35
Pre-admission antibiotics (%)	95.2	94.7	90	> 0.99
Days on antibiotics	5 (4-6)	4.0 ± 1.5	5.3 ± 1.8	0.01
Duration of symptoms at first dose of Oseltamivir, median (IQR) (days)	8 (7-10)	8 (7-10)	7 (5-8)	0.35
Comorbidities, %				
Obesity (BMI > 30 kg/m^2)	62.5	57.8	61.5	0.77
Diabetes mellitus	8.7	7.0	15.3	0.09
Asthma	5.7	7.0	0	0.57
Hypertension	10.1	14.0	0	> 0.99
Cardiac failure	1.4	1.7	0	> 0.99
Cirrhosis	1.4	1.7	0	> 0.99
Chronic pulmonary disease	7.2	8.7	0	0.57
Chronic renal disease	1.4	0	0	> 0.99
HIV/AIDS	1.4	3.5	0	0.99
Smoker	17.4	24.5	30.7	0.74
Alcohol/Substance abuse	13.0	17.5	15.3	0.84
Symptoms and signs, %				
Cough	100	100	100	> 0.99
Subjective fever	98.5	98.2	100	> 0.99
Sore throat	59.4	50.8	38.4	0.34
Sputum	41.5	82.4	79.2	0.45
Rhinorrhea	78.5	46.4	21.4	0.13
Hemoptysis	36.9	38.5	30.7	0.90
Dyspnea	100	100	100	> 0.99
Headache	67.7	86.6	92.3	0.05
Fatigue	100	100	100	> 0.99
Myalgia	84.6	85.9	76.9	0.68
Diarrhea	4.6	3.5	7.6	0.49
Cyanosis	52.1	32.6	84.6	0.04
Wheezing	15.4	19.2	0	0.10
Crackles	96.9	96.4	100	> 0.99

BMI: body mass index; IQR: interquartile range.

Table 2. Laboratory values, physiological response, severity scores on admission to the respiratory intensive care unit, and outcome of survivors and non-survivors

Variable	Total (n = 70)	Survivors (n = 56)	Non-survivors (n = 14)	p value
MAP (mmHg)	73 ± 11	74 ± 12	70 ± 7	0.61
HR (beats/minute)	91 ± 21	92 ± 20	88 ± 24	0.71
Vasopressors %	72.8	68.4	92.3	0.01
Norepinephrine dose (mcg/kg/min)	0.096 ± 0.065	0.088 ± 0.036	0.137 ± 0.066	0.02
Ratio of PaO_2 to FIO_2	95 ± 39	76 ± 30	60 ± 25	0.06
PEEP, cm H_2O	12 ± 4	12 ± 3	13 ± 3	0.26
Tidal volume adjusted to ideal weight, ml/kg	9.6 ± 2.8	9.4 ± 2.9	10.3 ± 2.3	0.27
Dynamic compliance	24.4 ± 5.0	25.1 ± 5.7	21.4 ± 3.8	0.02
Prone positioning ventilation, %	7.1	5.3	14.2	0.26
Corticosteroid therapy, %	90	87.5	100	
White blood cell count	10.7 ± 5.1	9.9 ± 4.8	13.8 ± 5.2	0.01
Lymphocyte count	897 ± 548	873 ± 451	996 ± 848	0.45
Procalcitonin (ng/ml), median (IQR)	0.88 (0.25-4.7)	0.54 (0.25-4.25)	2.4 (1.37-6.95)	0.38
Platelet count	218 ± 100	214 ± 98	232 ± 114	0.51
BUN		19.4 ± 13.0	32.7 ± 19.0	< 0.01
Creatinine (mg/dl), mean (SD)	0.88 ± 0.75	1.21 ± 1.02	2.01 ± 1.54	0.02
Bilirubin (mg/dl), median (IQR)	0.69 (0.5-1.10)	0.69 (0.5-1.0)	0.80 (0.6-1.35)	0.21
AST (U/l), median (IQR)	76 (40-109)	74 (38-108)	99 (67-110)	0.80
ALT (U/l), median (IQR)	48 (35-68)	50 (37-69)	40 (34-54)	0.44
Albumin (g/dl), mean (SD)	2.1 ± 0.45	2.16 ± 0.45	1.88 ± 0.42	0.04
INR, mean (SD)	1.12 ± 0.16	1.11 ± 0.16	1.15 ± 0.15	0.50
TTP (sec), mean (SD)	28.7 ± 4.3	28.5 ± 4.4	29.6 ± 3.8	0.50
LDH (U/l), mean (SD)	590 ± 760	597 ± 328	987 ± 442	< 0.001
Creatine kinase (U/l), mean (SD)	1,150 ± 967	1,376 ± 1,787	1,201 ± 1,049	0.727
SOFA score, median (IQR)	8 (5-9)	7 (4-8)	10 (8-11)	< 0.001
RICU length of stay (days), median (IQR)	18 (11-32)	19 (10-32)	18 (15-26)	0.53
Duration of mechanical ventilation, median (IQR) days	17 (8-29)	17 (7-29)	18 (15-26)	0.47
Ventilator-associated pneumonia, %	47.1	42.8	64.2	0.09
ICU-acquired weakness, %	81.4	82.1	78.5	> 0.99
Delirium, %	72.8	69.6	85.7	0.32

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; HR: heart rate; INR: international normalized ratio; IQR: interquartile range; LDH: lactic dehydrogenase; MAP: mean arterial pressure; PaO_2 to FIO_2: partial pressure of arterial oxygen to the fraction of inspired oxygen; PEEP: positive end-expiratory pressure; RICU: respiratory intensive care unit; SD: standard deviation; SOFA: Sequential Organ Failure Assessment; TTP: thrombotic thrombocytopenic purpura.

p = 0.02), although both groups represent a young population. There were no differences between survivors and non-survivors in distribution of comorbidities, time to seek primary medical attention, time from onset of symptoms to first dose of oseltamivir, time from onset of symptoms to hospital admission, or clinical manifestations and physical signs. However, the presence of cyanosis was more frequent among non-survivors (84.6 vs. 32.6%; p < 0.047). Obesity (body mass index, BMI > 30 kg/m^2) was the most frequent comorbidity and its prevalence was not different among survivors and non-survivors. There was no difference in the percentage of survivors and non-survivors who received antibiotics prior to hospital

admission (94.7 vs. 90%); however, non-survivors received antibiotics for a longer period of time (5.2 ± 1.9 vs. 4 ± 1.6 days; p = 0.022).

Cardiovascular features

Mean arterial pressure and heart rate were not different between survivors and non-survivors; however, a higher percentage of non-survivors required support with high doses of norepinephrine (92.3 vs. 68.4; p < 0.014). Non-survivors needed higher doses of norepinephrine during the first 24 hours to maintain mean arterial pressure (MAP) > 65 mmHg as compared to survivors (0.137 ± 0.066 vs. 0.088 ± 0.036 µg/kg/min; p = 0.022).

Respiratory function

Non-survivors had a lower PaO_2/FiO_2 ratio compared to survivors; however, this difference was not significant (60 ± 25 vs. 76 ± 30; p = 0.069). The required level of positive end-expiratory pressure (PEEP) and the tidal volume adjusted to ideal body weight were not different between the two groups. However, non-survivors showed lower dynamic compliance (non-survivors: 21.4 ± 3.8 vs. survivors: 25.1 ± 5.7; p = 0.027). A highly significant difference was observed in the levels of LDH between non-survivors and survivors (987 ± 442 vs. 597 ± 328; p = 0.000). Prone position ventilation was implemented in 5.3% of survivors and 14% of non-survivors (p = 0.260).

Renal function

The mean creatinine and blood urea nitrogen (BUN) values were significantly higher in non-survivors (Table 2). Acute kidney injury was present in 19 patients (27.1%), and of these, 15.7% were classified as AKI-1, 36.8% as AKI-2, and 47.3% as AKI-3. Hemodialysis was implemented in 77.7% of these patients. Mortality according to AKI classification was: no AKI or AKI-1: 0%; AKI-2: 28.5%; and AKI-3: 55.5%. Mortality in patients with AKI was 36.8% and for patients without AKI, 13.7%. AKI (2 or 3) was significantly associated with mortality in the univariate analysis (OR: 5.2; 95% CI: 1.4-18.5; p <0.01); however, it did not represent an independent risk factor in the multivariate analysis (OR: 1.7; 95% CI: 0.32-9.36; p = 0.512).

Infection features

Leukocyte count was higher in non-survivors and represented an independent risk factor for mortality. Procalcitonin levels were also higher in this group, although the difference was not statistically significant (0.54 [0.25-4.25] vs. 2.4 [1.37-6.95]; p = 0.386). However, in none of the cases was bacterial coinfection documented in the microbiological studies done within 48 hours of admission. Ventilator-associated bacterial pneumonia was present in 42.8% of survivors and 71.4% of non-survivors (p = 0.056). The most frequently isolated microorganisms were: *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Acinetobacter baumannii*, and *Staphylococcus aureus*. Other secondary infections were: invasive pulmonary aspergillosis, candidemia, *P. aeruginosa* and *S. aureus* bacteremia, and infected pressure sores.

Neurological manifestations

A high incidence of ICU-acquired weakness (ICU-AW, defined as a Medical Research Council [MRC] score[13] of < 48 points) was observed, and in a few cases, polyneuropathy and myopathy were documented by electromyography and nerve conduction studies. Delirium was observed in 87% of patients and was significantly associated with the length of stay in the RICU; however, there were no differences between the two groups.

Independent factors associated with mortality

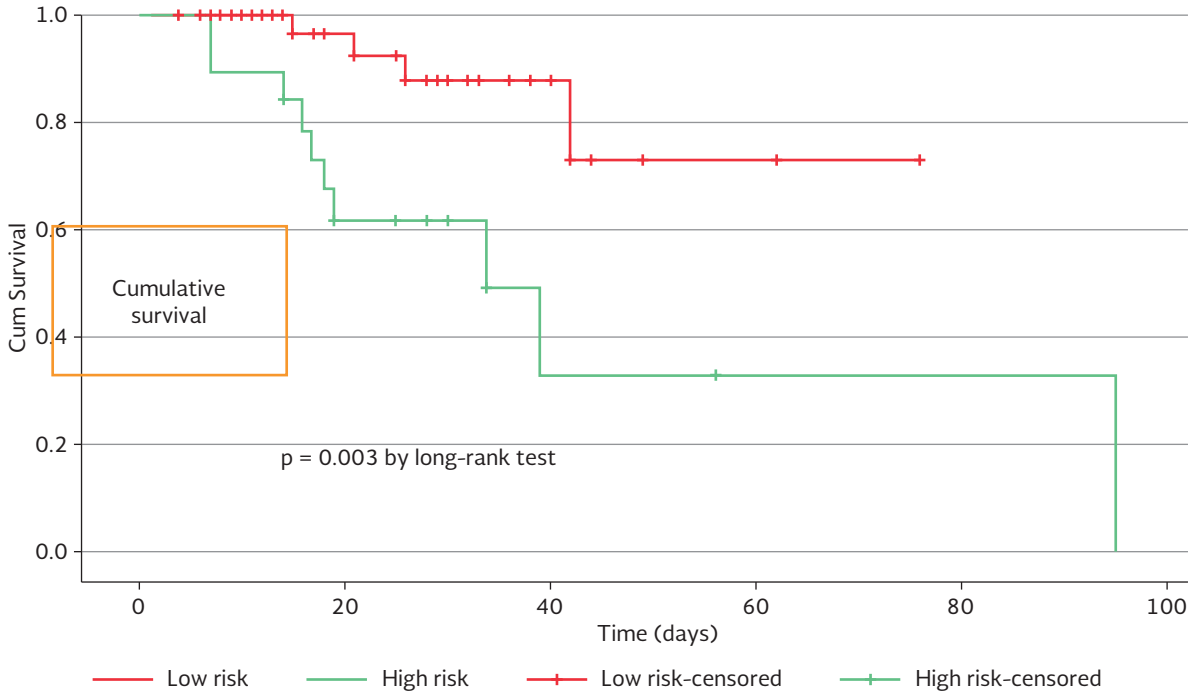
A logistic regression analysis was performed to ascertain the effects of age, white blood cell (WBC) count, norepinephrine dose in the first 24 hours, creatinine levels, and LDH levels on the likelihood of 90-day all-cause mortality. Of the five predictor variables, only three were statistically significant: age, WBC count, and LDH levels (Table 3). The logistic regression model was statistically significant, $\chi^2 = 27.402$, p < 0.0005. The model (Nagelkerke's R^2) explained 55% of the variance in the outcome variable and correctly classified 82.7% of cases. Sensitivity was 92.9%, specificity was 42.9%, positive predictive value was 60.0%, and negative predictive value was 86.6%. To test for a linear relationship between the continuous independent variables and the logit of the dependent variable,

Table 3. Results of multivariate logistic regression analysis and bootstrap

Variable	OR	95% CI	Bootstrap OR	95% CI
LDH	1.004	1.001-1.006	1.004	1.002-1.012
Age	1.102	1.019-1.191	1.102	1.032-1.321
WBC	1.222	1.046-1.428	1.222	1.051-1.900

LDH: lactic dehydrogenase; WBC: white blood cell count.

Figure 1. Survival curve in patients with acute respiratory distress syndrome secondary to influenza A(H1N1)pdm09 according to mortality score predictor. The cutoffs were: lactate dehydrogenase ≥ 700 U/l; age ≥ 55; white blood cell ≥ 12,000 cells/mm³. Patients with zero to 1 point were defined as having a low mortality risk, and patients with 2 to 3 points as high mortality risk. A highly significant difference between distributions of survival was observed (p < 0.001, by log rank test).



a Box-Tidwell approach was used, which adds an interaction term between an independent variable and its natural log to the equation. The interaction terms were not statistically significant. Increasing age, LDH levels, and WBC counts were associated with an increased likelihood of 90-day all-cause mortality. An un-weighted risk scale assigning one point to each risk factor was created using the independent risk factors. For purposes of the un-weighted risk scale, continuous variables were transformed into dichotomous variables by identifying the maximal sum of sensitivity and specificity. A score for each patient was then calculated. The cutoffs were: LDH ≥ 700 U/l; age ≥ 55; WBC ≥ 12,000 cells/mm³. Patients with zero to 1 point were defined as having a low mortality risk, and patients with 2 to 3 points as having a high mortality risk. A highly significant difference between distributions of survival was observed (p < 0.001, by log rank test) (Fig. 1).

DISCUSSION

In this study, we present the clinical characteristics and factors associated with mortality in 70 patients with influenza A(H1N1)pdm09 and ARDS admitted to our RICU during the 2013-2014 influenza season. This population was represented by young adults. Mortality at 28 days and at discharge from the RICU was 14% and 20%, respectively. This mortality compares

favorably with mortality reported during the influenza H1N1 pandemic of 2009 in Mexico[1,2].

Early administration of oseltamivir in patients with influenza has been reported to reduce the risk of complications and mortality. Rodriguez, et al. found that oseltamivir therapy initiated within two days of influenza symptoms was an independent variable associated with a significant reduction in mortality[14]. A recent meta-analysis reported that the risk of mortality increases 1.23 times for each day of delay in the initiation of treatment up to day 5[15]; furthermore, the interval between the onset of symptoms and initiation of antiviral therapy has been reported as an independent factor associated with increased severity of disease[14]. In our patients, the median time to first dose was eight days, reflecting a serious problem of logistics in primary health care services. Thus, the indiscriminate use of antibiotics as well as a failure in primary care attention probably contributed to the delay in hospital admission and the increase in severity of lung injury in our patients.

During the influenza pandemic of 1917-1918, most deaths were due to bacterial coinfection[16]. During the influenza pandemic of 2009, it was reported that bacterial coinfection had a significant prevalence that contributed to mortality[17]. However, Russell, et al. reported severe ARDS and multiple organ failure solely attributable to the A(H1N1)pdm09 virus[18]. Moreover, studies in animal models and in humans have demonstrated the ability of the influenza A(H1N1) pdm09 virus to trigger necrosis and apoptosis in the cells of the respiratory epithelium and alveolar pneumocytes, as well as an intense local and systemic cytokine response[19-21]. Thus, infection with the A(H1N1) pdm09 virus may be sufficient to cause severe lung injury, vasodilatory shock, and multiple organ failure in the absence of bacterial coinfection. In our patients, leukocytosis detected on admission was significantly associated with mortality, and procalcitonin levels were also higher in non-survivor patients, suggesting bacterial coinfection[22]. However, all cultures on admission were negative, suggesting unlikely bacterial coinfection, although it cannot be ruled out since almost all patients received one or more antibiotics before admission.

The prevalence of obesity in our patients was 65%, which is more than twice the prevalence of 28.1% reported for our country by the WHO[23]. This suggests an increased risk of infection with influenza A(H1N1) pdm09 virus in these patients, consistent with findings previously reported during the influenza pandemic of 2009[2,3]. Although an association between obesity and mortality in patients with influenza A(H1N1)pdm09 has been suggested[24,25], obesity was not associated with mortality in our cohort of patients. Our results agree with those reported by the Mexican and Canadian groups during the influenza pandemic of 2009[2,3] as well as with other studies showing that mortality in obese patients with ARDS is not increased[26].

We identified LDH as a strong independent mortality predictor. However, the source of this LDH cannot be determined with certainty because the LDH isoenzymes were not determined. Smith, et al. reported high levels of LDH in serum and bronchoalveolar lavage fluid in patients with pneumocystis pneumonia[27]. Their results suggest that the increase in serum levels of LDH is due to backflow of pulmonary derived LDH into the blood through a compromised alveolocapillary membrane. Thus, levels of LDH could reflect the severity of lung injury, and could prove to be a better predictor of mortality than the PaO_2/FiO_2 ratio in our cohort of patients. Our results are consistent with those reported by other researchers in patients with severe pulmonary viral infections[28,29].

A significant problem observed in our patients was a high incidence of early ICU-AW. The cause of this syndrome in our patients is probably multifactorial (obesity, steroids, neuromuscular blockage). However, myositis by the A(H1N1)pdm09 virus with necrosis of muscle fibers may have played a significant role. A history of severe myalgia and elevation of CPK is consistent with the diagnosis of viral myositis[30]. Cases of acute severe myositis by the A(H1N1)pdm09 virus have been reported[31], and the myotropism of the virus and its ability to produce muscle necrosis have been demonstrated in animal models and in humans[32,33]. Because the rapid course to ICU-AW was an important factor for prolonged mechanical ventilation, the early implementation of a physiotherapy program may be especially significant for limiting further muscle function impairment.

Acute kidney injury has been described as a complication in patients with influenza A(H1N1)pdm09 and an independent predictor for mortality. Bagshaw, et

al.[34] reported an incidence of 60.9% of AKI according to the RIFLE classification in 562 critically ill patients with H1N1 influenza; AKI was not an independent predictor for hospital mortality. In contrast, Martin-Loeches, et al.[35] reported an incidence of 17.6% in 660 critically ill patients, where AKI was an independent predictor for mortality. In our cohort, 27% of patients had AKI on admission, and mortality was significantly higher in those with AKI-3. However, in multivariate analysis, AKI did not represent an independent predictor for mortality. Early support with hemodialysis may have contributed to the reduction of mortality risk in this group.

We identified three factors independently associated with mortality. Based on these, we developed a score to classify the patients into strata of low and high risk of mortality. The internal model validation by the bootstrap method showed the stability of parameters. However, we were not able to conduct an external validation (gold standard). Although the results should be viewed with caution, they may be useful for stratifying the severity of a patient and may contribute to the decision-making process.

Our study had some limitations. First, results represent a single institution. Second, bias in patient selection cannot be ruled out since admission to the RICU depended on the judgment of the attending physician. Due to this selection bias, it is possible that patients with a more severe disease, and with an increased mortality risk, were not selected for admission to the RICU, which could have influenced the low mortality that we found compared with that reported in other institutions nationwide, although the severity of respiratory failure is similar to that reported in other centers. We think that the experiences gained in the pandemic and subsequent outbreaks and the care in a specialized unit are also important factors that influenced mortality, although a selection bias may be present. Third, although an internal validation of the predictive model was made, the gold standard is the external validation. Thus, the validity of the predictive factors for mortality derived from our cohort must be taken with caution; further validation with another data set is needed.

In conclusion, we described the clinical characteristics and course of a cohort of patients with ARDS secondary to influenza A(H1N1)pdm09, and developed a predictive model based on the covariates of age, levels of LDH, and WBC count on admission to the RICU. The model may be useful for stratifying the severity of the patient and may contribute to the decision-making process. However, external validation of this proposed model is still required.

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