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Excess mortality is associated with influenza A (H1N1) in patients with severe acute respiratory illness

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ABSTRACT

Background: Acute respiratory infections caused by viruses are among the leading causes of morbidity and mortality. The inflammatory response that follows viral infection is important for the control of virus proliferation. However, if overwhelming, may be associated with complicated outcomes.
Objectives: We assessed the clinical characteristics of patients with severe acute respiratory illness (SARI) evolving to acute respiratory distress syndrome (ARDS) and the factors related to death. Study design. Prospective study in 273 adult patients with SARI performed in a university-affiliated 800-bed hospital serving an area of epidemiologic vigilance of 102 municipalities and more than 2 million inhabitants. Influenza A (H1N1) 2009 (A/H1N1), influenza A H3N2, and influenza B were tested in all patients by RT-PCR.
Results: The overall hospital mortality rate was 17.6%. A total of 30.4% of patients tested positive for influenza A/H1N1. Patients with SARI that evolved to ARDS took significantly longer to take the first dose of oseltamivir (6.0 vs 1.0 days, $p = 0.002$). Patients with H1N1 positive tests had almost 3 times higher probability of death, despite having significantly less comorbidities ($p = 0.027$). The influenza A/H1N1 pdm09 vaccine reduced the odds of death by 78%. Nonsurvivors had a more intense inflammatory response than did survivors at 48 h (C-reactive protein: 31.0 ± 17.5 vs. 14.6 ± 8.9 mg/dl, $p = 0.001$) as well as a more positive fluid balance.
Conclusions: Hospital mortality associated with influenza H1N1-associated SARI and ARDS continued to be high years after the 2009 pandemic in a population with low vaccine coverage. Antiviral treatment started more than two days after onset of symptoms was more frequently associated with ARDS and death and, having had vaccine against influenza A (H1N1) was a factor independently related to survival.

1. Background

Acute respiratory infections caused by viruses are among the leading causes of morbidity and mortality around the globe, especially among older adults and those with chronic diseases. Viruses account for 40–50% of all causes of serious community-acquired pneumonia (CAP) [1,2]. Populations most susceptible to respiratory viruses include neonates, immunocompromised and elderly populations [3].

The influenza A (H1N1) 2009, after the first pandemic wave, continued to circulate with varying frequencies in various years and regions, causing serious CAP with acute respiratory failure (ARF). Studies in various countries pointed to circulation of other respiratory viruses in patients with severe acute respiratory illness (SARI), including human rhinovirus (HRV), respiratory syncytial virus (RSV) and adenovirus (AdV) [2–6]. In fact, more than twenty different viruses have been linked to CAP [7].

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The inflammatory response that follows viral infection is important for the control of virus proliferation, but if it is overwhelming, it may be associated with complicated outcomes such as bacterial coinfection, sepsis, acute respiratory distress syndrome (ARDS) and multiple organ failure [8]. In a large cohort of 178 patients with diffuse viral pneumonitis caused by the A/H1N1 virus admitted to the ICU in 2009, ARDS, organ failures and death were common [9]. ARDS complicates up to 55% of influenza-related pneumonia in hospitalized patients and carries a mortality of 40–46% [2].

The H1N1 influenza virus has a high recombination capacity and may be capable of provoking new pandemics with various clinical characteristics. Appropriate and early empiric antibiotic treatment has decreased mortality in patients with sepsis around the globe [10,11]. Our hypothesis was that worsening pulmonary function and mortality was related to influenza A/H1N1 infection and to the delay in the use of oseltamivir after the first flu symptoms in patients with SARI.

1.1. Objectives

Our primary objective was to identify clinical characteristics of patients with SARI evolving to ARDS and the factors associated with death. Secondary objectives were to evaluate the intensity of inflammatory response and aspects of fluid resuscitation.

1.2. Study design

This prospective cohort observational study was performed between January 1th and December 31th 2016 in a university-affiliated 800-bed hospital (Hospital de Base, São José do Rio Preto, SP, Brazil) serving an area of epidemiological vigilance of 102 municipalities and more than 2 million inhabitants. This study was approved by the Local Research Ethics Committee (CAAE:55720416.9.0000.5415).

All patients older than 16-year-old with SARI defined by the presence of an acute respiratory illness of recent onset (within the last ten days) manifested by fever ($\geq 38\,^{\circ}C$), cough and shortness of breath or difficulty in breathing requiring hospitalization with suspected influenza A H1N1 2009, were included in the study [11]. Samples of nasal and nasopharyngeal swabs, or tracheal aspirates in intubated patients, were collected to detect influenza A/H1N1 (2009) pdm, influenza A H3N2, and influenza B in all patients.

ARDS was defined according to the Berlin definition [12]. All patients with SARI undergoing mechanical ventilation were managed with a lung-protective ventilation strategy. Daily fluid balance (FB) was calculated as the difference between fluid administered and fluid lost [13]. Sequential organ failure assessment (SOFA) scores were determined at admission and at 24 and 48 h later [14]. All patients were followed-up for complicated outcomes and all-cause mortality during hospitalization.

Collected samples were processed by molecular methods (conventional polymerase chain reaction - PCR for HRV and AdV; quantitative real-time polymerase chain reaction – qPCR for influenza A and B and RSV). The nucleic acids were isolated directly from stored samples (maintained at $-80\,^{\circ}C$) to the PCR kits (DNA - QIAamp DNA Blood MiniKit, RNA - QIAamp Viral RNA Extraction Kit, Qiagen, Germany). After extraction the samples were stored at $-80\,^{\circ}C$ until PCR was performed. The primers used to amplify the nucleic acid of the studied viruses were previously described: influenza A and B [15,16], HRV [17], RSV [18], AdV [19]. Endogenous controls for RNA and DNA detection have been previously described [20,21]. The GoTaq® 1-Step RT-qPCR System commercial kit (Promega, USA) based on the SYBR green dye was used for qPCR test. For HRV detection the cDNA synthesis reaction was performed with Moloney Murine Reverse Transcriptase (MMLV-RT; Thermo Fisher, USA). Amplification of HRV cDNA and AdV DNA were performed with Platinum Taq DNA polymerase (Thermo Fisher, USA). For all molecular tests the manufacturer's instructions were followed.

Statistical analysis was performed with continuous variables reported as the mean ± standard deviation or median (25–75% interquartile range, IQR). The Kruskall-Wallis test was used for continuous variables due to the absence of a Gaussian distribution. Pearson's Chi-squared test or Fisher's exact test were used to compare categorical variables. Univariate and multivariate logistic regression (stepwise backward) were used to determine independent predictors of death. The independent variables used to adjust the model were: age (years), vaccination status, gender (reference: male), comorbidities (cardiovascular disease, lung disease, chronic kidney disease neurologic disease, immunosuppression), diabetes mellitus, obesity, fever, dyspnea and SpO2 lower than 95%. Variables yielding p-value < 0.25 by the univariate analysis and those considered clinically important were entered in a forward multivariate logistic regression analysis. The adjusted odds ratio (OR) and 95% confidence intervals (95% CIs) were calculated for the predictors.

2. Results

From January to December 2016, 273 patients with a clinical presentation of SARI and a test for influenza virus were included in this study. Of these, 20.5% had received seasonal influenza vaccine (influenza A (H1N1) pdm09). The mean age was 49.2 ± 17 years. There was a median of 3.0 days (2–7 days) from first symptom to the first dose of oseltamivir and 3.5 days (2–7 days) from first symptom to hospital admission. Eighty patients (29.3%) were treated with oseltamivir

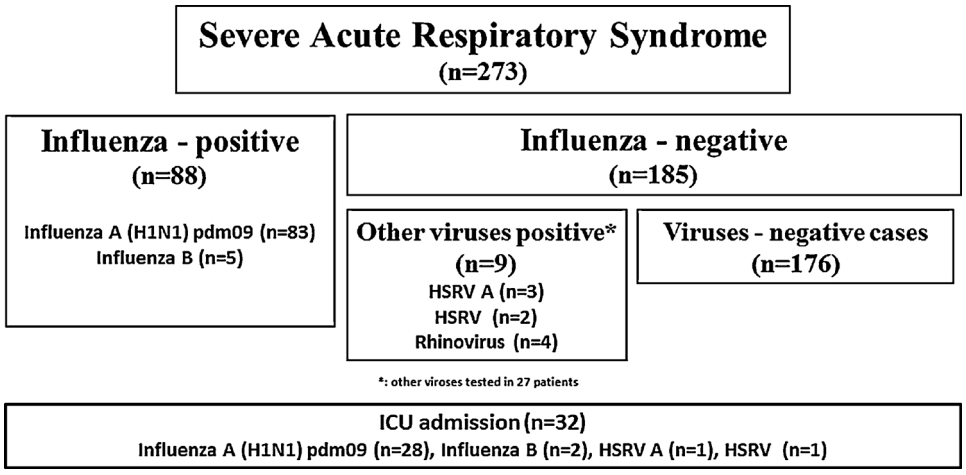

Fig. 1. Flow of patients admitted in the hospital with severe acute respiratory infection.

Table 1
Clinical characteristics of adult patients admitted in the hospital with severe acute respiratory infection in the influenza positive and influenza negative groups.

	Influenza positive (n = 88)	Influenza negative (n = 185)
Age. Years	48 ± 15	50 ± 18
Gender, Male	43 (49)	75 (40.5)
Pregnancy	3 (3.4)	5 (2.7)
H1N1 influenza virus vaccine		
No	51 (58.6)	109 (58.9)
Yes	13 (14.9)	43 (23.2)
Not known	23 (26.4)	32 (17.3)
Signs and symptoms		
Fever	76 (86)*	139 (75.1)
Cough	80 (91)	155 (84)
Dyspnea	63 (72)*	153 (83)
SpO2 < 95%	59 (67)	110 (60)
	76 (86)**	134 (72)
Chest X Ray findings at admission		
Interstitial	17 (19.3)	27 (14.6)
Consolidation	13 (14.8)	10 (5.4)
Mixed	10 (11.3)	21 (11.4)
Comorbidities		
None	63 (72)*	106 (57)
Obesity	11 (12.6)**	6 (3.2)
Diabetes mellitus	14 (15.9)	38 (20.5)
Immunossupression	12 (13.6)	26 (14.1)
Cardiovascular disease	12 (13.6)	41 (22.2)
Lung disease	1 (1.1)**	20 (10.8)
Liver disease	2 (2.3)	2 (1.1)
Neurologic disease	3 (3.4)	9 (4.9)
Chronic renal failure	4 (4.5)	16 (8.6)
Others	34 (38.6)	59 (31.9)
Outcomes		
Invasive mechanical ventilation	25 (28.4)	31 (16.8)
Non-invasive mechanical ventilation	34 (38.6)	73 (39.5)
ICU admission	36 (40.9)	56 (30.3)
Hospital LOS. days	6 [3-9]	6 [3–10]
Mortality rate	26 (29.5)**	22 (11.9)

Numbers are shown as n (%), means ± standard deviation, or median (interquartile range).
SpO2: Pulse Oximeter Oxygen Saturation; ICU: Intensive Care Unit; LOS: Length-of-stay.
* $p < 0.05$ vs. PCR negative.
** $p < 0.01$ vs. PCR negative.

within 48 h after the first symptoms.

The patients were divided into 2 groups according to the results summarized in Fig. 1. A total of 88 patients (32.2%) with positive tests for influenza were included in the PCR influenza positive group (influenza A/H1N1, n = 83; influenza B, n = 5). Three patients in this group had the first respiratory samples negative for influenza, but the tests were recollected and confirmed positive in postmortem samples. Human rhinovirus (RV), respiratory syncytial virus (RSV), and adenovirus (AdV) were tested in samples from 27 patients (9.9%) known to be negative for influenza. Demographics, clinical and laboratory data, and, the time interval between first symptom and the first dose of oseltamivir, were registered.

We found no positive tests for influenza H3N2. The PCR influenza negative group comprised the patients with negative tests (n = 185). Clinical characteristics and outcomes of the two groups are shown in Table 1. Only 15% of the patients in the PCR influenza positive group were vaccinated, and 72% of these were free of comorbidities in contrast to 57% in the group PCR-influenza negative patients (p = 0.024) (Table 1).

A total of 32 patients (12%) were admitted to the ICU due to worsening respiratory function, of whom two thirds (n = 21) evolved to ARDS. In patients admitted to the ICU, the time from first symptom to the first dose of oseltamivir was 4.0 days (CI 95% 3.5–5.5 days) and time from first symptom to hospital admission was 4.5 days (CI 95% 3.5–6.0 days). Patients with SARI who evolved to ARDS took significantly longer to take the first dose of oseltamivir and to be admitted to the hospital (median time; 6.0 vs 1.0 days, p = 0.002) than did patients who did not evolve to ARDS. Fig. 2 shows times from symptoms to first dose of oseltamivir in patients with and without ARDS. Differences between patients with and without ARDS are shown in Table 2. SOFA scores were significantly higher in patients with ARDS.

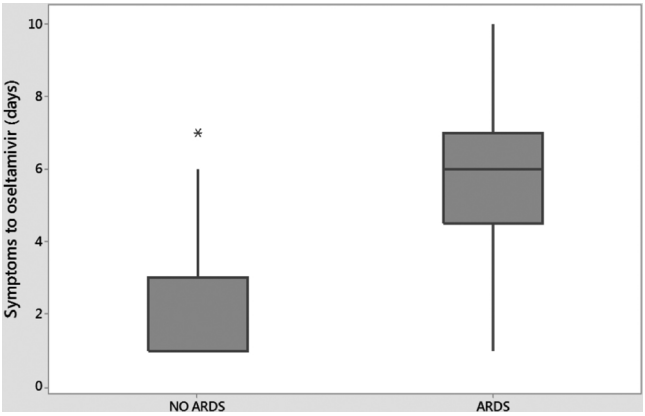

Fig. 2. Box-Whisker-Plot displaying time from onset of symptoms to first dose of oseltamivir in patients with severe acute respiratory infection with and without Acute Respiratory Distress Syndrome (ARDS).

The overall hospital mortality rate was 17.6%, 12.5% for patients treated with oseltamivir within 48 h and 19.7% for those treated after 48 h (p = 0.156). The mortality rate was significantly higher in patients with PCR influenza positive than in PCR influenza negative (29.5% vs. 11.9%, p < 0.001) (Table 1). In patients with H1N1 positive tests, median time from first symptoms to the first dose of oseltamivir was 5 days [2–6.5 days] for nonsurvivors and 3 days [2–4 days] for survivors (p = 0.026).

The independent predictors of death in the binary logistic regression are shown in Table 3. C-reactive protein levels were significantly higher in nonsurvivors than in survivors (day 1, 22.3 ± 16.2 mg/dl vs. 10.5 ± 9.9 mg/dl, p = 0.003, day 2; 31.0 ± 17.5 vs. 14.6 ± 8.9 mg/dl, p = 0.001) Fig. 3. ARDS was diagnosed in 82% of the patients who died and 47% of the patients who survived (p = 0.032). Interval plots of means and 95% confidence intervals of the SOFA score in survivors and in nonsurvivors during 3 days in ICU are shown in Fig. 4. Need for renal replacement therapy was more frequent in nonsurvivors (53%) than in survivors (13%) (p < 0.05). There was a steady increase in daily fluid balance in both survivors and nonsurvivors, with nonsurvivors having a significantly more positive fluid balance at day 4 (survivors: 498 mL [-757-1793 mL]; nonsurvivors: 1875 mL [1774–2350 mL], p = 0.027) Fig. 5.

Nine out of a sample of 27 influenza negative patients tested for others viruses had the following positive tests; RSV A (n = 3), RSV B (n = 2) or RV (n = 4). Coinfection with bacterial pathogens was microbiologically confirmed in 3 patients admitted to the ICU in less than 48 h after admission (tracheal aspirates positive for Penicillin-*susceptible Staphylococcus aureus* in 2 cases and *Pseudomonas aeruginosa* in one). In further 6 patients the following pathogens were isolated from tracheal aspirates more than 48 h after admission: *Pseudomonas aeruginosa* (n = 2), *Acinetobacter baumanni* (n = 2), *Klebsiella pneumonia* (n = 1), and, *Staphyloccocus aureus*, (n = 1).

3. Discussion

The main results of our study were as follows. First, high hospital

Table 2
Characteristics and outcomes of the patients admitted in the ICU with severe acute respiratory infection associated or not with acute respiratory distress syndrome (ARDS).

ARDS	All (n = 32)	Yes (n = 21)	No (n = 11)	P value
Age. years	46 ± 16	48 ± 16	43 ± 17	0.606
Time 1 st symptom / admission	5.0 [1.25 -7.0]	6.0 [4.5-7.0]	1.0 [1.0-3.0]	0.002
Time 1 st symptom / oseltamivir	5.0 [1.0 – 7.0]	6.0 [4.0-7.0]	1.0 [1.0-3.0]	0.003
H1N1 influenza virus vaccine	2 (6.0)	0 (0)	2 (18.2)	0.110
SOFA day 1	7 [4–11]	11 [5–12]	4.0 [2.0-7.0]	0.025
SOFA day 2	9 [3–11]	10.0 [7–12]	3.0 [2.0-5.0]	0.012
SOFA day 3	9 [5–11]	10 [8–11]	3.5 [0.7-5]	0.023
Serum lactate (mEq/L) day 1	1.7 [1.2-2.6]	2.0 [1.4-2.7]	1.4 [1.0-2.1]	0.212
Serum lactate (mEq/L) day 2	1.9 [1.1-2.3]	2.0 [1.4-2.4]	1.0 [0.9-4.4]	0.276
Serum lactate (mEq/L) day 3	1.6 [1.1-2.1]	1.7 [1.2-2.2]	1.3 [0.9-2.0]	0.606
CRP (mg/dL) day 1	12.9 [4.9-30.1]	13.5 [3.9-30.1]	8.2 [5.3-26.6]	0.853
CRP (mg/dL) day 2	20.4 [9.2-34.2]	24.0 [9.0-41.4]	17.5 [10.4-24.7]	0.531
CRP (mg/dL) day 3	14 [7.8-21.1]	16.7 [8.7-21.4]	10.1 [5.9-20.0]	0.370
Leucocytes, day 1	9750 [4280-13285]	7080 [3908-12793]	12020 [9830-13385]	0.144
Leucocytes, day 2	7770 [4200-15410]	9280 [4563-16235]	5390 [1570-7770]	0.180
Platelets, day 1	166 [107-238]	150 [109-241]	175 [85-245]	0.888
Platelets, day 2	158 [123-290]	157 [119-286]	158 [121-298]	0.741
P/F, day 1	122 [79-273]	102 [62-184]	350 [284-409]	0.003
P/F, day 2	146 [101-251]	130 [99-189]	313 [280-417]	0.012
Creatinine (mg/dL), day 1	1.1 [0.9-1.3]	1.0 [0.82-1.35]	1.2 [0.95-2.15]	0.367
Creatinine (mg/dL), day 2	1.1 [0.8-3.3]	1.15 [0.90-2.82]	0.80 [0.50-4.55]	0.508
MV, days	15.5 [4.2 -21]	15 [5–30]	16 [2.5-7.5]	0.255
MV free days	0 [0-1.25]	0 [0-0.5]	1 [0-4]	0.184
Shock	22 (69)	18 (86)	4 (36)	0.004
VAD, days	5 [3–6]	5.0 [3.0-6.2]	4.5 [1.50-6.25]	0.666
RRT	11 (34)	3 (27)	8 (38)	0.536
ICU LOS, days	9 [5.2-24.5]	14.0 [6.0-29.0]	6.0 [3.0-17.0]	0.059
Hospital LOS, days	16 [6–28]	13 [6.0-31.0]	17 [6.0-20.0]	0.563
Mortality rate	21 (100)	14(66.6)	7(33.3)	0.032

Number are presented as n (%), median ± standard deviation or median [25%–75%].
ARDS: Acute Respiratory Distress Syndrome; SOFA: Sequential Organ Failure Assessment; CRP: C reactive protein;P/F: PaO2/FiO2 ratio; MV: Mechanical ventilation; VAD: vasoactive drugs; RRT: Renal Replacement Therapy, ICU: Intensive Care Unit; LOS: Length-of-stay.

mortality persisted in influenza A/H1N1-associated SARI years after the 2009 pandemic in a population with a low vaccination coverage. Second, earlier use of oseltamivir was associated with lower mortality rates. Third, vaccine decreased the likelihood of death by almost 80%, but only 20% of the patients with H1N1 were vaccinated, and, 72% of these patients were free of comorbidities. Finally, nonsurviving patients had a more severe inflammatory response and a more positive fluid balance.

Delayed antiviral treatment increased lung involvement and mortality. Hypoxemia reported as a low SpO2 at hospital admission occurred in 62% of the patients with SARI, increasing the odds of death by more than a factor of 14. Patients with SARI who evolved to ARDS or died took a significantly longer time to take the first dose of oseltamivir. ARDS was diagnosed in 82% of the nonsurvivors and in 47% of the survivors. Patients with ARDS more frequently had shock (86%), higher mortality (53%) and higher serum lactate levels.

The mortality rate associated with Influenza A/H1N1 remains high, despite advances in critical care in the last decade. In our study, patients who were influenza positive had almost two times the risk of death than did other patients with SARI. Interestingly, despite the higher mortality, influenza positive patients had fewer comorbidities and were younger than influenza negative patients. Accordingly, using a multivariable model, a Canadian study in patients with 2009 pandemic influenza (H1N1) showed that the likelihood of death increased almost 3 times for every 2 days of treatment delay after the first symptom, and this factor was most strongly associated with the severity of the disease [22]. In a large cohort study, antiviral treatment and hospital admission delays were significantly associated with the risk of death among influenza A/H1N1 inpatients after adjusting for age, gender, geographic region and pandemic wave [23]. Indeed, a randomized study confirmed that the use of oseltamivir for more than 5 days after illness onset did not reduce clinical failures among hospitalized patients with influenza-associated lower respiratory tract infections [24]. One Brazilian study showed 81% protection among individuals

Table 3
Binary logistic regression analysis with independent predictors of death in patients with severe acute respiratory infection.

	Coefficient ± Standard error	OR	CI 95%	P value
H1N1 influenza virus vaccine	−1.550 ± 0.748	0.212	0.054-0.823	0.010
Gender (Male)	0.861 ± 0.402	2.365	1.076-5.200	0.029
H1N1(pdm) 09	1.021 ± 0.413	2.776	1.235-6.240	0.013
SpO2 lower than 95% at hospital admission	2.666 ± 0.653	14.28	3.968-51.40	< 0.001
Obesity	−1.550 ± 0.748	10.04	2.453-41.15	0.001
Immunosuppression	1.299 ± 0.513	3.657	1.338-10.00	0.013
Cardiovascular disease	1.125 ± 0.463	3.074	1.241-7.611	0.016

OR: Odds ratio; CI 95%: Confidence interval. Goodness-of-Fit Tests (Hosmer-Lemeshow: Chi-square -3.63 p = 0.726).
SpO2: Pulse Oximeter Oxygen Saturation.

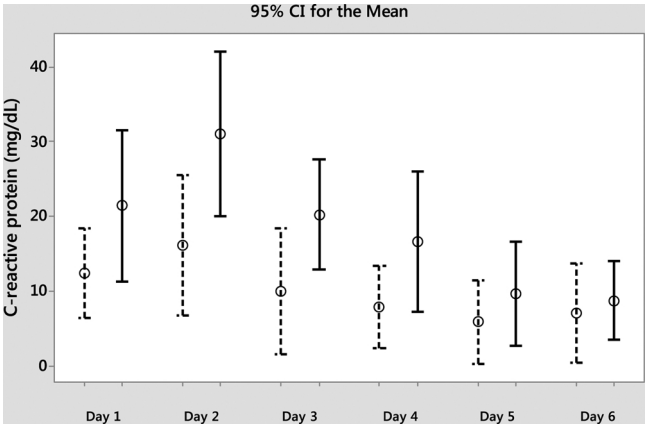

Fig. 3. Interval plots of means and 95% confidence interval of C-reactive protein in survivors (dotted line) and in nonsurvivors patients (continuous line) during 6 days in ICU patients with severe acute respiratory infection.

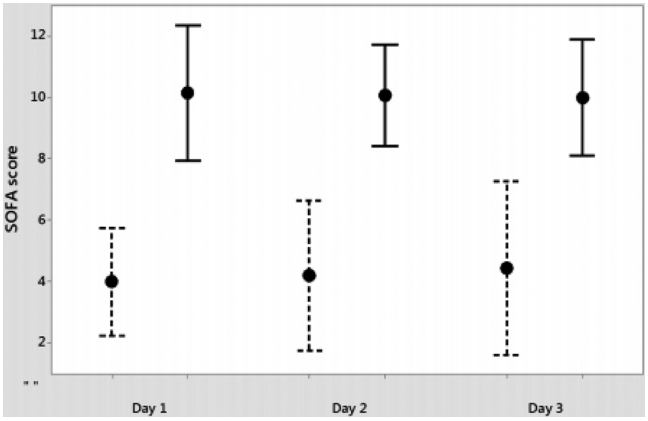

Fig. 4. Interval plots of means and 95% confidence interval of SOFA score in Survivors (dotted line) and in Non-survivors patients (continuous line) during 3 days in ICU patients with SARI.

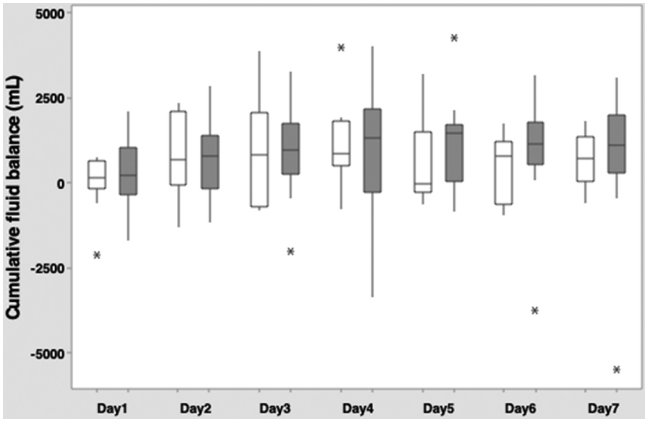

Fig. 5. Box-Whisker-Plot displaying cumulative fluid balance in the first week in survivors (white) and nonsurvivors (gray).

who received treatment within the first 48 h during the 2009 pandemic and that after 72 h, there was no significant protection [25]. These findings have important clinical implications in view of the continuous impact of influenza A/H1N1 and other viruses causing SARI and fatalities in recent years.

Vaccination might reduce the risk of influenza-like illnesses [26]. The studied population had low vaccination coverage, probably contributing to a higher hospital mortality rate. Other authors described the protective effect of vaccine against seasonal influenza in 2009 [26–29]. Seasonal influenza vaccination in our cohort appeared to reduce the severity of the disease and the likelihood of death by almost 80%. A Canadian study suggested that the adjuvant influenza A H1N1 pdm09 vaccine prevented approximately 55%–60% of pneumonia- and influenza-related hospitalizations among children and younger adults, 10%–15% of hospitalizations among 65 or older adults and 14% of those with pre-existing chronic diseases [29]. Our data suggested that there is a need for wider vaccination coverage during influenza campaigns, not only in the more vulnerable groups, because 70% of the patients with H1N1/SARI had no comorbidities.

Several viruses have been described as causative agents of SARI [30,31]. It appears that viral pathogens are increasingly recognized as a cause of pneumonia in immunocompetent patients, reinforcing the importance of surveillance for other viral etiologies in patients with SARI [32]. Indeed, in a small sample of 27 patients tested negative for influenza we found RSV in 5 patients and RV in 4 patients. In addition, there is a lack of studies investigating bacterial coinfections or secondary infections in patients suspected of influenza A H1N1 pdm09 infection. In our study, bacterial growth was tested in all 32 patients admitted to the ICU of whom 9.3% had positive tests early after ICU admission and 18.7% later than 48 h after admission. Positive bacterial growth ranged from 0 to 47% (mean 19%) in different studies on influenza A H1N1, however the rate of bacterial co-infection may be underestimated in these studies as well as in ours as many cases are not tested for bacterial infections, and bacterial pneumonia is not always easy to be diagnosed in patients with viral infections, particularly, critically ill patients [33].

It appeared that the clinical presentations, laboratory findings and patterns of risk factors for influenza A/H1N1-associated SARI have not changed considerably in the waves after 2009. Of note, patients who died experienced a more intense inflammatory response than did those discharged home. In a prospective observational study in heterogeneous populations of ICU patients, higher serum C-reactive protein concentrations at ICU admissions were independently associated with initial acute respiratory failure or development of organ failure and death [34]. In patients with 2009 H1N1 influenza infection in China, C-reactive protein levels were also significantly higher in nonsurvivors than in survivors [35].

Nonsurvivors had significantly more positive fluid balance than did survivors, with a positive fluid balance of more than 3 liters at day 5. Increases in the extravascular lung water and positive fluid balance have been associated with acute kidney injury (AKI) and death in critically ill patients [36,37]. One retrospective study suggested that the rapidity of fluid accumulation was associated with AKI and mortality in patients with severe pneumonia due to influenza A/H1N1 [35]. In patients with influenza A/H1N1 associated-SARI, a negative fluid balance within 7 days of admission was significantly associated with a lower risk of death [38]. Attention should be paid to fluid balance in these patients.

We and other authors [26,27] found that, on logistic regression analysis, male gender and comorbidities such as cardiovascular disease, immunosuppression, diabetes and obesity were independent predictors of death. Obesity, probably due adipokine-induced immunodysregulation, and immunosuppressive therapy emerged as new risk factors for severe influenza after pandemic influenza A/H1N1(2009) [39]. A study including 34,493 admissions for laboratory-confirmed influenza during waves from 2005 to 2012 found that male gender, cardiovascular disease, diabetes, obesity and immunosuppression, among others, were predictors of mechanical ventilation, death or both [40].

Our study has some limitations, primarily related to the monocentric characteristics of the study, the sample size and the inclusion of patients with a clinical diagnosis of SARI with negative tests for influenza. We acknowledge that studies based on registry databases are vulnerable to bias, however the prospective design and the inclusion of all patients in a 1-year cohort from a large area of epidemiologic

vigilance of more than 2 million inhabitants might decrease selection bias. Another limitation is that, due to limited resources, only a few viral tests were performed in a small number of patients in the Influenza-negative patients, making significant comparison to other viruses difficult.

In conclusion, hospital mortality associated with SARI, particularly influenza H1N1-associated SARI and ARDS, continued to be high in a population with low vaccine coverage. Antiviral drug treatment started more than two days after onset of symptoms was more frequently associated with ARDS in patients with SARI and having had vaccine against influenza A (H1N1) was a factor independently related to survival.

Conflict of interest

On behalf of all authors, the corresponding author states that is no conflict of interest.

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CRediT authorship contribution statement

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