Outcome of influenza A (H1N1) patients admitted to intensive care units in the Paraná state, Brazil

Pacientes com infecção por vírus A (H1N1) admitidos em unidades de terapia intensiva do Estado do Paraná, Brasil

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ABSTRACT

Objective: This study aimed to analyze outcome, clinical and epidemiological characteristics and severity factors in adult patients admitted with a diagnosis of infection by virus A (H1N1) to public and private intensive care units, in Paraná, Brazil.

Methods: Cohort study of medical charts of patients older than 12 years admitted to 11 intensive care units in 6 cities in the state of Parana, Brazil, during a period of 45 days, with diagnosis of swine influenza. The diagnosis of infection with A (H1N1) was made by real time polymerase chain reaction (RT-PCR) of nasopharyngeal secretion, or strong clinical suspicion when other causes had been ruled out (even with negative RT-PCR). Descriptive statistics were performed, analysis by the Chi square test was used to compare percentages and the Student's t test for continuous variables with univariate analysis, assuming a significance level of $p < 0.05$.

Results: There were 63 adult patients admitted with a diagnosis of H1N1, 37 (58.7%) being RT-PCR positive. Most patients were young adults (65% under 40 years of age) with no gender predominance and high incidence of obesity (27.0% with Body Mass Index > 30). Mean of the Acute Physiologic Chronic Health Evaluation II (APACHE II) score was 15.0 + 8.1. Mortality in the intensive care unit was 39.7%. The main factors associated with mortality were: positive RT-PCR, low levels of initial PaO2/FiO2, high initial levels of urea and lactate dehydrogenase, required level of positive end expiratory pressure, need for the prone position and vasopressors.

Conclusions: Adult patients with A (H1N1) virus infection admitted to intensive care units had a high risk of death, particularly due to respiratory impairment. Positive RT-PCR, urea and lactic dehydrogenase, low initial PaO2/FiO2 and high levels of PEEP were correlated with higher mortality.

Keywords: Influenza A virus; Intensive care units; Respiration, artificial.

INTRODUCTION

In March-April 2009,cases of acute respiratory syndrome associated to influenza were described in Mexico and the United States and identified as a new swine influenza A virus.[1] This was a new virus, genotipically different from other A viruses: avian H1N1, swine or human (responsible for the Spanish Influenza in 1918). It quickly spread through the North Hemisphere within the next weeks, reaching Europe in May.

On June 11, 2009 the World Health Organization (WHO) raised the in-

fluenza A: H1N1 epidemic level to the maximum alert level of 6, officially declaring the world in a new influenza pandemic, which was considered "uncontrollable".[2]

The H1N1 pandemic reached Parana, Brazil early in June 2009. Shortly after, Parana became one of the states with the highest incidence and mortality rates of the disease in Brazil, apparently because of its frontier with Argentina, a country that along with Chile had the first South American H1N1 cases in May (end of fall in the Southern hemisphere).

This study aimed to observe and analyze Influenza A: H1N1 cases in patients aged above 12 years admitted to 11 Intensive Care Units (ICU) in 6 different cities in the Parana State, Brazil, for 45 days.

METHODS

This was a cohort observational study with all patients above 12 years of age diagnosed with acute A: H1N1 virus infection admitted to an adult ICU in 11 hospitals of 6 cities in the state of Parana (Southern Brazil) evaluated from July 18, 2009 to August 21, 2009.

Patients' medical records and clinical epidemiologic information, plus ICU admission laboratory tests, oxygen therapy and mechanic ventilation data, along with ICU and hospital course were evaluated.

The Influenza A:H1N1 diagnostic testing was performed by the Real Time Polymerase Chain Reaction (RT-PCR) using oropharyngeal secretion - "Kit Superscript III Platinum One-Step Quantitative RT-PCR System"* (Invitrogen, Carlsbad, USA).

This test was performed at the Parana State Health Secretariat Central Laboratory. The inclusion criteria were: (1) patients with RT-PCR virological diagnosis or (2) patients clinically diagnosed (based on clinical-epidemiologic data), however with a negative RT-PCR test. In RT-PCR negative cases, clinical diagnosis was reached by excluding other conditions such as seasonal influenza and other viruses, or bacterial pneumonia.

Descriptive statistics and Chi square percentual comparisons were performed, and the quantitative variables were compared using the Student's t test, with a $p<0.05$ significance level. Analysis was univariated. The study was approved by the Universidade Estadual do Oeste do Paraná Ethics Committee.

RESULTS

During the study period there were 574 admissions in the 11 studied ICUs (55.7% in private hospitals and 44.3% in public or philanthropic hospitals), of which 63 patients (11.0%) with influenza H1N1 infection were included. The patients' epidemiological data are shown on table 1. The mean age was 35.0 years, and 46.1% were male. Of the H1N1 patients, 42.9% were treated in pri-

Table 1- Clinical-epidemiologic data of patients with H1N1 diagnosis admitted to the intensive care unit (n=63)

Variable	Total (N=63)	RT-PCR pos (N=37)	RT-PCR neg (N=26)	P value
Male	46.0	48.6	47.8	0.847
Age (years)				
13 to 25	23.8	24.3	17.4	0.730
26 to 40	41.3	45.9	34.8	0.535
41 to 55	20.6	16.3	30.4	0.308
≥ 56	14.3	13.5	17.4	0.946
Time (days) from the onset of symptoms to ICU admission	6.7± 3.70	7.27 ± 3.58	6.22 ± 3.82	0.269
Comorbidities	42.8	48.6	39.1	0.625
Obesity (BMI >30)	27.0	32.4	21.7	0.518
COPD	9.5	10.8	8.7	0.878
DM	3.2	2.7	4.3	0.717
CHF functional class III or IV	4.8	5.4	4.3	0.695
CRF plus dialysis	0	0	0	
Neoplasms	1.6	2.7	0	0.857
HIV	1.6	2.7	0	0.857
SLE	1.6	2.7	0	0.857
Current pregnancy	12.7	10.8	17.4	0.704
APACHE II first ICU 24h	15.0 ± 8.11	16.2 ± 7.84	15.4 ± 8.64	0.704

ICU - intensive care unit; COPD - chronic obstructive pulmonary disease; BMI - body mass index; DM - diabetes mellitus; CHF - congestive heart failure; CRF – chronic renal failure; HIV - human immunodeficiency virus; SLE- systemic lupus erythematosus; APACHE - Acute Physiology and Chronic Health Evaluation. Results expressed as mean ± standard deviation or %.

vate ICUs, and 57.1% in public ICUs. The mean time from the onset of symptoms to ICU admission was 6.0 days. All patients underwent RT-PCR nasopharyngeal secretion testing, which was positive in 37 (58.7%) patients. Invasive mechanic ventilation was required for 71.4%.

The admission laboratory tests and clinical features are shown in table 2.

The ICU mortality was 39.7%, while of those under mechanic ventilation (MV) it was 53.3%. There was no difference in mortality between private and public ICUs (36.1% vs 44.4%, p=0.685).

Eight of the patients were pregnant (12.7%), including one in the 3rd post-partum day. Maternal mortality in the ICU was of 25%.

There was no age-related difference in mortality (x^2=5.09; p=0.16).

The main ICU mortality related factors (Table 3) were positive RT-PCR test, initially low arterial oxygen pres-

Table 2 –Clinical-epidemiological and laboratory characteristics at intensive care unit admission (n=63)

Variable	Total N=63	RT-PCR pos N=37	RT-PCR neg N=26	P value
Leucocytes (Cells x 103/mm3)	9.89 ± 7.57	7.28 ± 3.34	14.22 ± 10.59	<0.005
Platelets (Cells/mm3)	172.0 ± 98.92	162.9 ± 79.58	196.6 ± 123.16	0.192
LDH(UI/ml)	504.7 ± 591.4	1067.2 ± 668.9	504.7 ± 270.3	<0.001
CPK (UI/ml)	694.6 ± 1324.1	856.9 ± 1335	694.6 + 252	0.544
Baseline Creatinine (mg/dl)	1.26 ± 1.25	1.28 ± 1.17	1.27 ± 1.46	0.976
Creatinine > 1.5 mg/dl	17.4	18.9	17.4	0.858
Arterial Lactate	2.44 ± 2.34	2.32 ± 4.72	2.28 ± 6.81	0.978
Baseline PaO2/FiO2	150.0 ± 91.23	119.4 ± 79.36	189.4 ± 102.6	0.003
Baseline PaCO2 (mmHg)	40.7 ± 20.4	38.5 ± 13.2	46.7 ± 27.94	0.124
Higher PEEP / first 12 h	15.1 ± 7.29	16.9 ± 7.37	12.7 ± 6.29	0.021
VD use in the first 04h	42.8	43.2	47.8	0.917

RT-PCR - Real time - Polymerase Chain Reaction; LDH – Lactic dehydrogenase; CPK - Creatino-phosfokynase; PEEP – Positive end expiratory pressure; VD - Vasopressor drugs. Results expressed as mean ± standard deviation or %.

Table 3 – Mortality risk factors

	Outcome		P value
	Alive (N=38)	Dead (N=25)	
Epidemiological data			
Male	50.0	36.0	0.404
Age > 50 years	26.0	16.0	0.531
Comorbidities	28.9	32.0	0.985
Obesity (BMI>30)	23.0	32.0	0.617
Pregnant	18.0	8.0	0.455
Admission laboratory tests			
RT-PCR Positive	48.6	80.0	0.01
LDH > 1.5 x Normal	34.0	64.0	0.038
Leucocytes (cells x 103/mm3)	9.8 ± 10.3	9.9 ± 5.2	0.95
Platelets (cells x 103/mm3)	176.2 ± 116.9	169.3 ± 86.6	0.79
Lactate	5.51 ± 5.41	3.84 ± 5.67	0.29
Creatinine (mg/dl)	1.37 ± 1.32	1.18 ± 1.20	0.56
Urea (mg/dl)	52.90 ± 45.87	34.68 ± 21.26	0.04
Clinical/respiratory data			
Use of vasopressor drugs	31.6	72.0	0.004
PEEP > 15 cmH2O Needed	26.3	68.0	0.003
PaO2/FiO2	96.83 ± 57.80	175.01 ± 89.48	<0.005
PaCO2 (mmHg)	45.10 ± 26.40	37.81 ± 14.89	0.17
Use of prone Position	10.5	44.0	0.006
NIMV Use	39.4	24.3	0.319
APACHE II > 20	23.6	40.0	0.268
Positive fluid balance*	28.9	48.0	0.203

LDH – Lactic Dehydrogenase; SD – Standard Deviation; NIMV – non-invasive mechanic ventilation; APACHE - Acute physiology and chronic health evaluation. * % patients with >1800ml/24 hours fluid balance on the first ICU day. Results expressed as mean ± standard deviation or %.

Table 4 – Clinical outcomes

Variable	Total N=63	RT-PCR pos N=37	RT-PCR neg N=26	P value
Invasive mechanic ventilation needed	45 (71.4)	27 (73.0)	20 (76.9)	0.955
ICU mortality	25 (39.7)	20 (54.0)	5 (19.2)	0.012

ICU – intensive care unit. Results expressed as numbers (%).

sure/inspired oxygen fraction rate (PaO_2/FiO_2), initially increased urea and lactic dehydrogenase (LDH), positive end-expiratory pressure (PEEP) required , need for prone position and use of vasopressor drugs.

Table 4 displays the outcomes of positive and negative RT-PCR results.

DISCUSSION

In March 1918 (during World War I) a severe influenza epidemic spread throughout the world, beginning simultaneously in the U.S. and Europe and killing (particularly in the "second wave", that started 5 months later) about 40 million people, becoming the most catastrophic medical event ever in human history.[3] The strain involved was Influenza A (H1N1) from birds. Since then, the influenza virus surveillance systems had identified changes in the A virus epidemic strains every 1 to 2 years (by surface glycoprotein mutations, hemagglutinin and neuropeptidase). In the last 91 years, two A virus pandemics were identified: in 1957 (H2N2) and 1968 (H3N2), although they were much less lethal.[4-6] During the last years, with epidemics observed such as severe acute respiratory syndrome (SARS), the imminence of an influenza pandemic has been alerted by the health authorities.[3,7,8]

In this trial, it was seen that 11.0% of ICU admissions were due to H1N1 cases. An important bias is that these hospitals were mostly considered as reference for treatment of H1N1 patients, which could increase disease incidence. Another important factor to bear in mind is that in, at least one hospital, a special unit was created for H1N1 patients. Thus, in practice the number of ICU beds increased during this period (as the original ICU continued to take care of non-H1N1 patients). Finally, there were instructions to reduce the elective surgeries in public hospitals as a health system strategy, which could temporarily reduce some indications for ICU admissions.

The ICU mortality in this study was high, particularly among invasive MV patients. This finding is also found in literature. In 30 of the admitted cases (either with virological or clinical diagnosis) in California hospitals (USA), six patients were admitted to the ICU (4 under mechanic ventilation), with no death (although three were still hospitalized at the reporting time).[9]According to Perez-Padilha et al.,[10] of the 18 H1N1 cases confirmed in patients with respiratory failure, 10 under mechanical ventilation were reported; of the total of patients under mechanical ventilation (MV), 70% died.

The main mortality risk factors identified were related to clinical severity, particularly respiratory impairment. Another trial found these main factors associated to higher risk of mortality: hypotension requiring vasoactive drugs, acute renal failure, metabolic acidosis, APACHE II, PaO_2/FiO_2 and Sequential Organ Failure Assessment (SOFA) admission score.[10] The severe respiratory impairment is similar to severe influenza cases, just as SARS.[11]

As previously described in literature,[10] ICU mortality was higher among positive RT-PCR test patients than among those with a negative test. This could reflect a higher viral load and morbidity in positive RT-PCR patients, although as mentioned below, this could also mean that among the RT-PCR negative patients other diagnoses, with milder courses could be included. Another possibility is inappropriate material collection, which could reflect in increased false-negatives rates.[12]

Pregnancy is acknowledged as a risk factor for respiratory complications in infections by influenza.[13,14] Among the factors justifying the high incidence in this group, physiological changes of pregnancy, including reduced pulmonary functional residual capacity and cell-mediated immunity impairment, are emphasized.[15] However, in patients with severe infection requiring ICU stay, the mortality rate is similar to that of the general population.[9] In our study the maternal mortality among pregnant women was similar to the overall group (25.0% vs 41.8%; p=0.602).

Obesity has clearly been incriminated as a severity and mortality risk factor among swine influenza patients.[16] It is believed that this is due both to the disease-related respiratory effects (reduced pulmonary functional residual capacity) and presence of typically associated comorbidities (such as diabetes, asthma, cardiovascular diseases, etc).[9] Obesity was very frequent among this group of patients,

similar to literature: 27.0% had a body mass index (BMI) above 30.

The time from onset of the symptoms and ICU admission was relatively long (6 days). In the Spanish trial,[16] time from onset of symptoms and beginning of treatment was 4 days.

This study has limitations. The total number of cases may have been overestimated. In our study, were considered as H1N1 cases, not only patients with confirmed laboratory diagnosis, but also those with strong clinical-epidemiological suspicion, although with negative RT-PCR. According to the Health Ministry of Brazil,[17] "influenza A:H1N1 will be discarded if influenza A virus is not detected by RT-PCR or culture techniques." However, although RT-PCR is the World Health Organization (WHO) and Center for Disease Control (CDC) recommended method,[12,18] false-negative results reach 10%.[19,20] Thus, the investigators decided to assess the strongly suspected cases (clinical diagnosis), even though with a negative influenza A test (since other causes were discarded). This was the same methodology as that used in previous reports,[9,10] although in the Spanish trial[16] only positive cases were accepted for diagnosis. However, the authors recognize that this approach constitutes a significant limitation for this study regarding overall data interpretation.

The univariate analysis may have been a restricted tool to define mortality-associated risk factors and therefore a limitation for interpretation of this study data.

CONCLUSIONS

Adult patients with an influenza A(H1N1) diagnosis admitted to an ICU have an increased mortality rate. The main mortality predictive factors found in this trial were: severe respiratory impairment, positive RT-PCR test, increased baseline urea and LDH, and need for vasopressor drugs. However, diagnostic methods (including clinical diagnosis) and the reduced number of patients may have contributed to jeopardize interpretation of this study data.

RESUMO

Objetivo: Analisar a evolução, características clínico-epidemiológicas e fatores de gravidade em pacientes adultos admitidos com diagnóstico de infecção por vírus A(H1N1) em unidades de terapia intensiva públicas e privadas no estado do Paraná, sul do Brasil.

Métodos: Estudo coorte de análise de prontuários de pacientes com idade superior a 12 anos admitidos em 11 unidades de terapia intensiva de 6 cidades no estado do Paraná (Brasil), durante um período de 45 dias, com diagnóstico de gripe suína. O diagnóstico de infecção por vírus A(H1N1) foi feito através de *real time -polimerase chain reaction* (RT-PCR) da secreção nasofaríngea, ou de forte suspeita clínica quando descartadas outras causas (mesmo com RT-PCR negativo). Foi feita estatística descritiva e análise com teste chi quadrado, para comparação entre porcentagens e teste t de student para variáveis continuas, com análise univariada, admitindo-se como significante um $p<0,05$.

Resultados: Foram admitidos 63 pacientes adultos com diagnóstico de H1N1, sendo 37 (58,7%) RT-PCR positivos. A maioria dos pacientes era de adultos jovens (65% com idade inferior a 40 anos), sem predominância de sexo e alta incidência de obesidade (27,0% com índice de massa corpórea>30). A média do escore *Acute Physiologic Chronic Heatlh Evaluation* II (APACHE II) foi de 15,0 ± 8,1. A mortalidade na unidade de terapia intensiva foi de 39,7%. Os principais fatores associados a essa mortalidade foram exame positivo no teste RT-PCR, níveis baixos de relação PaO_2/FiO_2 inicial, níveis elevados de uréia e desidrogenase lática iniciais, nível de pressão expiratória final positiva necessária, necessidade de posição prona e de drogas vasopressoras.

Conclusões: Pacientes admitidos em unidades de terapia intensiva com infecção por vírus A(H1N1) apresentaram alto risco de óbito, particularmente devidos ao comprometimento respiratório. O exame RT-PCR positivo, níveis de uréia e de desidrogenase láctica, além baixa PaO2/FiO2 e necessidades de PEEP alta, foram relacionados com uma maior mortalidade.

Descritores: Vírus da influenza A; Unidade de terapia intensiva; Ventilação mecânica

REFERENCES

1. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, Garten RJ, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med. 2009;360(25):2605-15.
2. World Health Organization. Global Alert and Response (GAR). DG Statement following the meeting of the Emergency Committee [Internet]. [cited 2009 Sep 29]. Available from: http://www.who.int/csr/disease/swineflu/4th_meeting_ihr/en/index.html .
3. World Health Organization. Global Alert and Response (GAR). Avian influenza: assessing the pandemic threat [In-

ternet]. [cited 2009 Sep 29]. Available from: www.who.int/csr/disease/influenza/WHO_CDS_2005_29/en/ .

4. Kamps BS, Hoffmann C, Preiser W, editors. Influenza report 2006 [Internet]. Paris: Flying Publisher; 2006. 225 p. [cited 2009 Sep 29]. Available from: http://www.influenzareport.com/influenzareport.pdf
5. Morens DM, Taubenberger JK, Fauci AS. The persistent legacy of the 1918 influenza virus. N Engl J Med. 2009;361(3):225-9. Erratum in: N Engl J Med. 2009;361(11):1123.
6. Zimmer SM, Burke DS. Historical perspective - Emergence of influenza A (H1N1) viruses. N Engl J Med. 2009;361(3):279-85
7. World Health Organization. Director-General. Pandemics: working together for an effective and equitable response. [Internet]. [cited 2009 Sep 13]. Disponível em: http://www.who.int/dg/speeches/2007/20070613_seattle/en/index.html.
8. Lazzari S, Stöhr K. Avian influenza and influenza pandemics. Bull World Health Organ. 2004; 82(4):242.
9. Centers for Disease Control and Prevention (CDC). Hospitalized patients with novel influenza A (H1N1) virus infection - California, April-May, 2009. MMWR Morb Mortal Wkly Rep. 2009;58(19):536-41.
10. Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, Hernandez M, Quiñones-Falconi F, Bautista E, Ramirez-Venegas A, Rojas-Serrano J, Ormsby CE, Corrales A, Higuera A, Mondragon E, Cordova-Villalobos JA; INER Working Group on Influenza . Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in Mexico. N Engl J Med. 2009;361(7):680-9.
11. Phua GC, Govert J. Mechanical ventilation in an airborne epidemic. Clin Chest Med. 2008;29(2):323-8, vii.
12. World Health Organization. CDC protocol of realtime RTPCR for influenza A (H1N1). [Internet]. [citado 2009 Set 13]. Disponível em: http://www.who.int/csr/resources/publications/swineflu/CDCRealtimeRTPCR_SwineH1Assay-2009_20090430.pdf.
13. Fiore AE, Shay DK, Broder K, Iskander JK, Uyeki TM, Mootrey G, Bresee JS, Cox NS; Centers for Disease Control and Prevention (CDC); Advisory Committee on Immunization Practices (ACIP). Prevention and control of influenza: recommendations of the Advisory Committee on Immunization Practices (ACIP), 2008. MMWR Recomm Rep. 2008;57(RR-7):1-60.
14. Centers for Disease Control and Prevention (CDC). Novel influenza A (H1N1) virus infections in three pregnant women - United States, April-May 2009. MMWR Morb Mortal Wkly Rep. 2009;58(18):497-500. Erratum in: MMWR Morb Mortal Wkly Rep. 2009;58(19):541.
15. Rasmussen SA, Jamieson DJ, Bresee JS. Pandemic influenza and pregnant women. Emerg Infect Dis. 2008;14(1):95-100.
16. Rello J, Rodriguez A, Ibanez P, Socias L, Cebrian J, Marques A, Guerrero J, Ruiz-Santana S, Marquez E, Del Nogal-Saez F, Alvarez-Lerma F, Martinez S, Ferrer M, Avellanas M, Granada R, Maravi-Poma E, Albert P, Sierra R, Vidaur L, Ortiz P, Prieto Del Portillo I, Galvan B, Leon-Gil C, H1N1 Semicyuc Working Group I. Intensive care adult patients with severe respiratory failure caused by Influenza A (H1N1)v in Spain. Crit Care. 2009;13(5):R148.
17. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Gabinete Permanente de Emergências em Saúde Pública. Emergência de Saúde Pública de Importância Internacional - ESPII. Protocolo de manejo clínico e vigilância epidemiológica da influenza. Portal da Saúde [Internet]. [citado2009 Set 13]. Disponível em: http://portal.saude.gov.br/portal/arquivos/pdf/protocolo_de_manejo_clinico_05_08_2009.pdf.
18. Centers for Disease Control and Prevention. Interim guidance on case definitions to be used for investigations of novel influenza A (H1N1) cases. [Internet]. [cited 2009 Sep 13]. Disponível em: http://www.cdc.gov/h1n1flu/casedef.htm.
19. Ellis J, Iturriza M, Allen R, Bermingham A, Brown K, Gray J, Brown D. Evaluation of four real-time PCR assays for detection of influenza A (H1N1)v viruses. Euro Surveill. 2009;14(22):pii:19230.
20. Ginocchio CC, Zhang F, Manji R, Arora S, Bornfreund M, Falk L, et al. Evaluation of multiple test methods for the detection of the novel 2009 influenza A (H1N1) during the New York City outbreak. J Clin Virol. 2009;45(3):191-5.