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Epidemiological Profile of Influenza A Cases in Southern Brazil in the Post-Pandemic Period

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Abstract

Influenza viruses are highly contagious and circulate in all geographical regions. During the 2009 pandemics caused by influenza A(H1N1), the State of Rio Grande do Sul (RS) was the first to detected A(H1N1) cases. In 2010, a broad vaccination program was applied in RS when 44.9% of the population joined the program. During the 2011, a total of 1,433 samples were sent to the Central Laboratory in Porto Alegre (LACEN-RS) for viral detection by qRT-PCR. Only 107 (7.5%) cases of the A(H1N1) virus were confirmed versus 182 (12.7%) cases of seasonal influenza A. The incidence of both influenza types virus was higher in patients aged 0-10 years old. The median viral load was higher in patients infected with seasonal, in comparison to those infected with A(H1N1) virus contrary of pandemic period. In 2011 most of the patients that were infected by influenza A virus (79%, p<0.001), did not receive vaccine. The presence of fever, cough, dyspnea, myalgia and rhinorrhea were the most frequent symptoms (positivity >60%). Furthermore in 2011 only patients infected by pandemic virus died (12.9%, p=0.001) in contrast with 2009 pandemic period when 6% of patients infected by pandemic virus died. In other hand in the whole population (5.3%) the mortality rate was similar that observed in pandemic period (5.9%). These analyses about epidemiological and molecular data provide important scenery about the characteristics of the host-pathogen interaction after massive exposure during pandemic period.

Introduction

Influenza viruses are highly contagious and circulate in all geographical regions. Although it causes mild symptoms in the majority of cases, illnesses can result in hospitalizations and deaths mainly among high-risk groups (the very young, elderly or chronically ill patients). Worldwide, these annual epidemics result in about three to five million cases of severe illness, and about 250,000 to 500,000 deaths [1].

In spite of the constant concern of authorities and the establishment of health surveillance systems, influenza A viruses are known for its tendency to antigenic variation, which can be quick and unpredictable [2]. Intense selection from the host immune system drives antigenic change in influenza virus, resulting in continuous replacement of circulating strains [3]. In Brazil, during the 2009 pandemics caused by influenza A(H1N1)pdm09 virus, over 80,000 cases of severe acute respiratory infection (SARI) cases were reported, among which 27,850 cases were confirmed as pandemic influenza. Hospitals and health care services were overcrowded and unprepared for such a large number of cases, especially in the South, where 18,349 cases were confirmed and mortality rates reached 2.32 (per 100.000 inhabitants); the State of Rio Grande do Sul (RS) alone confirmed 2,109 influenza A(H1N1) pdm09 case [4]. In 2010, a massive vaccination program was applied in RS when 4,898,723 people (44.9% of the population) were vaccinated. As a consequence, the A(H1N1)pdm09 virus was not detected in 2010 in RS, but reemerged in 2011 and has since then been increasingly circulating in the population, together with seasonal H3N2 influenza. Co-circulation of viral subtypes has been observed since the pandemic period [5]. Past the pandemic period, after August of 2010, the behavior of influenza A(H1N1)pdm09 virus is like that of a seasonal virus [1].

Brazil has a Program of Surveillance of Influenza since 1999 and has as goal the identification of the viral subtype, including emergent strains. Meanwhile, investigation of biological and clinical aspects, such as viral loads and correlation with aspects of the host is needed for the continuity of influenza A virus control, prevention and treatment of infected patients. Concerned with these issues, our group has started a continuous research on the molecular epidemiology of influenza A in RS, combining molecular analyses based on qRT-PCR, genome sequencing of viral samples, quantitation of viral loads, with clinical data of patients. Accordingly, we investigated influenza A cases in the State of Rio Grande do Sul during the year of 2009, showing that viral loads were much higher in patients infected by the pandemic influenza A (H1N1)2009 than with seasonal influenza A virus; furthermore, relative viral load correlated positively with symptoms such as chills, myalgia and rhinorrhea [5]. Viremia in patients infected by different influenza A subtypes depends on several factors that interfere with

virus–host interaction; mutations that lead to antigenic changes, the host's immunity and genetic background, as well as vaccination status are factors that contribute to the virus fitness and influence viral loads.

In this study we analyzed the relative viral loads observed in patients infected with either A(H1N1)pdm09 virus or seasonal influenza A viruses in the State of Rio Grande do Sul, Southern Brazil, during 2011 and correlate the results with the clinical and demographical data in the post- pandemic period.

Materials and Methods

Study subjects, clinical data and biological samples

During the epidemiological weeks 1-52 (January-December) of 2011, 1,501 cases of severe acute respiratory infection were reported in the State of Rio Grande do Sul (RS), Southern Brazil (estimated population: 10.7 million people). Of these, nasopharyngeal aspirate samples were collected from 1,433 patients, together with the *Influenza Notification Form* (demographic characteristics, date of notification, date of onset of symptoms, acute respiratory infection symptoms, co-morbidities, smoking habits, pregnancy status, vaccination status, and X-ray results). The form is filled out by the attending physician/nurse, at time of collection at the respective health unit, however some data might be neglected.

All 1,433 samples and forms were sent to the State Central Laboratory (LACEN-RS) for real time reverse transcription-polymerase chain reactions (qRT-PCR) analysis. Samples were identified either as pandemic influenza A or seasonal influenza A according to the qRT-PCR results (see below). All experiments were performed in compliance with relevant laws and institutional guidelines and in accordance with the ethical standards of the Declaration of Helsinki. The Research Ethics Committee of UFCSPA approved this study.

Identification and quantitation of influenza A virus

RNA was extracted from 1,433 nasopharyngeal aspirate samples using the QIAamp Viral RNA Mini kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. qRT-PCR was performed using the SuperScript-III Platinum One-Step Quantitative kit and the Influenza A (H1N1) Primer and Probe Set (Invitrogen-Life Technologies, Carlsbad, CA) as described elsewhere. Briefly, reactions were performed using 0.5 ml of SSIII/Platinum Taq Mix, 1 mM of each primer, 250 nM of probe, 12.5 μl 2X Master Mix, 5 μl of RNA sample and water, to a final volume of 25 μl. This method is based on a panel of 4 primers/probe sets: InfA is designed for universal detection of type A influenza viruses; swInfA detects all swine influenza A viruses; swH1 detects swine H1 influenza; and RNP targets the human RNase P gene and serves as an internal positive control for human nucleic acid. Therefore, if a sample was amplified with all the sets, it was considered pandemic influenza A. Samples that were positive only with InfA and RNP were considered seasonal influenza A. Samples that amplified only with RNP were considered negative for influenza A [5]. All PCR reactions were performed in a Step One Plus thermocycler (Applied Biosystems-Life Technologies, Carlsbad, CA). The following reaction conditions were applied: 50°C for 30 min; 95°C for 2 min; 45 cycles at 95°C for 15 s and 55°C for 35 s.

The relative viral load in each sample was calculated with the threshold cycle (CT) value based on the $2^{-\Delta CT}$ method [6]. The CT values obtained in the qRT-PCR with the InfA and RNP primer/probe sets (target and reference genes respectively) were used for delta calculation ($\Delta CT = CT_{InfA} - CT_{RNP}$).

Statistical analysis

Only samples with complete epidemiological data (n=88 for influenza A(H1N1)pdm09 and n=149 for seasonal influenza A) were included in statistical analyses to compare viral loads and clinical data from patients infected by pandemic or seasonal influenza. Regarding clinical conditions such as co morbidities, smoking habit and disease evolution, the total number of patients analyzed was 80 and 120 for pandemic and seasonal influenza A, respectively.

Descriptive statistics was used to summarize the data. Categorical data were treated with chi- square and Exact Fisher test, while continuous variables were compared using Mann–Whitney test, Md (P25-P75). Values were considered statistically significant when P<0.05.

Results

Influenza A identification by qRT-PCR

In 2010, the first post-pandemic year in Brazil, no cases of influenza A(H1N1)pdm09 virus were confirmed. Nevertheless, the virus reemerged in May 2011 and has since then circulated in the human population. Accordingly, 1,501 cases of SARI were notified in 2011 in RS. Of these, 1,433 nasopharyngeal aspirates were collected and sent to the Central Laboratory in Porto Alegre (LACEN-RS) for viral detection by qRT-PCR. A total of 107 (7.5%) cases of influenza A(H1N1)pdm09 virus were confirmed versus 182 (12.7%) cases of seasonal influenza A. Further analyses showed the seasonal influenza A being of the H3N2 subtype (data not shown). The highest influenza activity occurred in June, with concomitant circulation of both A(H1N1)pdm09 and H3 subtypes. The seasonal influenza was detected during epidemiological weeks 1-32, with five positive outbreaks in different institutions. The A(H1N1)pdm09 circulation persisted until the epidemiological week 44, especially after the seasonal influenza decline.

Samples negative for influenza A (n=1,144) were investigated for other respiratory viruses. Respiratory Syncytial Virus accounted for 20.1% of the influenza-negative SARI cases, and influenza B, adenovirus and parainfluenza (1, 2 and 3) viruses accounted for a total of 4.4% of these cases. In 67% of SARI cases, no respiratory viruses were found.

Characteristics of patients infected with influenza A in rio grande do sul

Complete *Influenza Notification Forms* were available for 88 and 149 pandemic and seasonal influenza A confirmed cases, respectively. However, clinical conditions such as comorbidities, smoking habit and disease evolution (death) were available only for 80 pandemic and 120 seasonal influenza-infected patients.

As shown in Table 1, the age distribution of patients infected by pandemic and seasonal influenza A in the year 2011 was homogenous. Among patients infected with A(H1N1)pdm09 virus, 53.4% were male and among patients infected with seasonal virus, 52.3% were female.

The frequency of pandemic and seasonal influenza A infection varied significantly among age groups (p=0.01), and the highest frequency of both subtypes was in patients aged 0-10 years (39.8% and 27.3%, respectively). When the distribution of the type of influenza in each age group was compared, the highest difference between seasonal

and pandemic influenza was in patients in the age group 31-40 years old (14.1% and 4.5%, respectively) (Table 1 and Figure 1). When age groups that are more susceptible to infection (0-10 years old and ≥ 60 years old) are combined, a total incidence of about 46.7% is observed for both A(H1N1)pdm09 and seasonal influenza A viral infection.

Regarding the vaccination status of the patients, in the year 2011 most of the patients that were infected by influenza A virus (79%, p<0.001) did not receive vaccine (Table 1).

All samples examined in this study were from patients presenting SARI with at least one of the symptoms: fever, cough, chills, dyspnea, sore throat, arthralgia, myalgia, conjunctivitis, rhinorrhea, diarrhea, as depicted in Table 2. The presence of fever, cough, dyspnea, myalgia and rhinorrhea were the most frequent symptoms both in patients infected by pandemic as well as by seasonal influenza A (positivity >60%) and conjunctivitis was the least frequent symptom, presented by 6.7% and 15.6% of patients with seasonal and pandemic influenza A, respectively. Notably, fever, dyspnea and conjunctivitis showed a positive correlation with infection by A(H1N1)pdm09 virus (p<0.05).

Characteristic	Seasonal (n=149)[a]	Pandemic (n=88)[a]	p
Age group (years)			
0-10	35 (27.3%)	35 (39.8%)	*p=0.01
11-20	13 (10.2%)	11 (12.5%)	
21-30	12 (9.4%)	13 (14.8%)	
31-40	18 (14.1%)	4 (4.5%)	
41-50	8 (6.3%)	11 (12.5%)	
51-60	17 (13.3%)	8 (9.1%)	
61-70	16 (12.5%)	6 (6.8%)	
71-80	7 (5.5%)	0	
81-90	2 (1.6%)	0	
Gender			
Female	78 (52.3%)	41 (46.6%)	*p=0.235
Male	71 (47.7%)	47 (53.4%)	
Viral load			
(Median [p25-p75])	1.86 (0.06-157.58)	0.05 (0.002-2.44)	#p<0.001
Vaccine			
Yes	33 (28.9%)	8 (9.5%)	*p=0.001
No	81 (71.1%)	76 (90.5%)	

[a]Vaccination status was not informed for all patients
*Statistically significant by Chi-Square Test
#Statistically significant by Mann Whitney U Test

Table 1: Demographic characteristics of patients infected by seasonal and pandemic Influenza A virus.
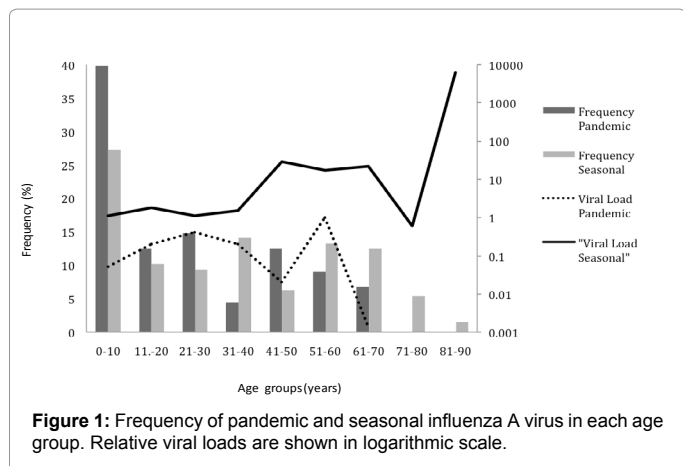
Figure 1: Frequency of pandemic and seasonal influenza A virus in each age group. Relative viral loads are shown in logarithmic scale.

Symptom	Frequencies		
	Seasonal (n=149)[a]	Pandemic (n=88)[a]	p Seasonal/Pandemic
Fever			
Yes	120 (93.8%)	87 (100%)	*p=0.017
No	8 (6.2%)	0	
Cough			
Yes	125 (97.7%)	85 (98.8%)	p=0.532
No	3 (2.3%)	1 (1.2%)	
Chills			
Yes	55 (50.4%)	36 (50%)	p=0.952
No	54 (49.6%)	36 (50%)	
Dyspnea			
Yes	90 (72%)	73 (84.9%)	*p=0.028
No	35 (28%)	13 (15.1%)	
Sore Throat			
Yes	40 (37.1%)	28 (41.2%)	p=0.583
No	68 (62.9%)	40 (58.8%)	
Arthralgia			
Yes	42 (39.6%)	22 (33.8%)	p=0.449
No	64 (60.4%)	43 (66.2%)	
Myalgia			
Yes	64 (60.9%)	45 (66.2%)	p=0.487
No	41 (39.1%)	23 (33.8%)	
Conjunctivitis			
Yes	8 (6.7%)	12 (15.6%)	*p=0.045
No	111 (93.3%)	65 (84.4%)	
Rhinorrhea			
Yes	72 (64.9%)	50 (62.5%)	p=0.670
No	49 (35.1%)	30 (37.5%)	
Diarrhea			
Yes	22 (20.2%)	15 (18.5%)	p=0.999
No	97 (79.8%)	66 (81.5%)	

[a]Not all symptoms were available for all patients
*Statistically significant by Chi-Square Test

Table 2: Frequencies of acute respiratory infection symptoms in patients infected by pandemic and seasonal influenza A virus.

In addition to symptoms attributable to SARI, other clinical manifestations that represent risk factors to this acute respiratory disease were observed in patients infected with both seasonal and pandemic virus. The frequency of each symptom was lower than the frequency of SARI symptoms with cardiopathy and pneumopathy being the most frequent comorbidities observed (Table 3). The distribution between the two types of virus was homogenous.

Only patients infected by the pandemic virus died (12.9%, p=0.001), showing an overall frequency of 5.4%.

Relative viral load and demographic data

In order to compare data from the post pandemic periods with that obtained during the pandemic period according to variables such as age, gender, clinical condition or severity of symptoms, viremia was calculated for each patient. Analysis or relative viral loads in seasonal and pandemic influenza A samples was based on the $2^{-\Delta CT}$ method [6], considering the CT values obtained in qRT-PCR with the infA primer/probe set [5].

The median relative viral load was higher in patients infected with seasonal virus in comparison to those infected by A(H1N1)pdm09 virus (1.86[0.06-157.58] vs. 0.05[0.0020-2.44], p<0.001) (Table 1).

When median viral load was compared between different age

groups for pandemic or seasonal influenza cases, no differences were observed (Table 4). On the other hand, the highest viral loads for pandemic influenza occurred in the age group 41-70 years, followed by the age group 21-30 years (Figure 1); regarding seasonal influenza, the highest viral loads occurred in patients older than 70 years, followed by the age groups 41-50 years and 61-70 years (Table 4).

The median viral loads between females and males infected by seasonal influenza A virus were statistically different, with females displaying higher viral loads in relation to male (11.72[0.15-340.35] versus 0.81[0.02-47.3], p=0.02). As for patients infected by pandemic influenza A, no significant differences were found in viral loads between genders.

Viral load did not show statistically significant association with any respiratory infection symptom. Nevertheless, considering other clinical conditions such as immunosuppression and metabolic disease, a positive association between high viral loads and these conditions was observed within seasonal influenza group only (Table 4).

Discussion

Past the pandemic period when a new influenza A virus was introduced worldwide, a strong surveillance was applied to monitoring the circulation of respiratory viruses in Brazil. Prevention actions coordinated by the Ministry of Health to contain the flu, including fast diagnosis and treatment of patients, were boosted all over the country, especially in the state of Rio Grande do Sul (RS), where high incidence of SARI is observed. A massive vaccination program was applied in RS beginning in April 2010, when 44.9% of the population was vaccinated;

| Characteristic | Viral Load | | |
	Seasonal (n=128) Md (P25-75)	Pandemic (n=88) Md (P25-75)	*P* Seasonal/Pandemic
Gender			
Male	0.81 (0.02-47.3)	0.1(0.0005-1.9)	*p=0.02/0.86
Female	11.72 (0.15-340.35)	0.04 (0.007-3.4)	
Age group (years)			
0-10	1.1 (0.2-90.6)	0.05(0.03-8.0)	#p=0.1/0.49
11-20	1.9 (0.4-1280.0)	0.2 (0.0005-10.5)	
21-30	1.1 (0.1-71.3)	0.4 (0.01-3.7)	
31-40	1.5 (0.02-170.2)	0.2 (0.03-0.1)	
41-50	28.8 (0.5-411.8)	0.02 (0.0006-0.1)	
51-60	17.1 (0.4-1676.5)	1.0 (0.02-5.4)	
61-70	21.6 (1.1-524.2)	0.0013 (0.0-2.0)	
71-80	0.6 (0.2-0.9)	-	
81-90	6238.3 (891-.)	-	
Comorbidity			
Immunosuppression			
Yes	438.6 (30.8-12495.9)	0.114 (.0001-14.9)	#p=0.049/0.87
No	1.87 (0.06-163.2)	0.101 (0.003-3.3)	
Metabolic disease			
Yes	1503.9 (8.2-18024.6)	4.23 (3.53-.)	#p=0.025/0.175
No	1.9 (0.07-168.9)	0.089 (0.002-2.7)	

*statistically significant by Chi-Square Test
Results by Mann Whitney U Test

Table 4: Viral loads in patients infected by pandemic and seasonal influenza A virus distributed by gender and age group. Statistically significant clinical conditions are also shown.

in that year, which was the first post-pandemic year in Brazil, no cases of influenza A virus were confirmed. Nevertheless, the virus reemerged in May 2011 and since then both A(H1N1)pdm09 and seasonal influenza A have been co-circulating with influenza B virus.

In the present study, virologic and epidemiological data was described for 237 patients who were diagnosed with pandemic (n=88) and seasonal (n=149) influenza A viruses infection in 2011. Analyses combining epidemiological and molecular data provide important information about the disease, including characteristics of the host-pathogen interaction after massive exposure during pandemic periods. The viral loads, vaccine and epidemiological data for patients infected by influenza A viruses during the whole year were analyzed. Nasopharyngeal aspirates were collected from patients in RS presenting with symptoms of acute respiratory infection and tested by qRT-PCR. Only 7.5% cases of A(H1N1)pdm09 virus were confirmed versus 12.7% of seasonal influenza A (either H1N1 or H3N2). In the last publication describing the 2009 pandemics in southern Brazil found that, among patients presenting SARI, 30% were positive for A(H1N1)pdm09 and 5.5% were positive for seasonal influenza [5]. According to Dapat et al. [7] decrease in the number of clinical cases may be attributed to an increase in antibody levels against the A(H1N1)pdm09 virus in the community. In the case of Brazil, such a decrease can also be attributed to the vaccination campaign.

In the present study, the frequency of infection varied significantly among age groups. The highest frequency of infection was observed in patients between 0 and 10 years old in both seasonal (27.3%) and pandemic (39.8%) groups. This is quite different from the pandemic period, in which the highest frequency was in the age groups 21-30 years old (26.3% seasonal and 25.7% pandemic influenza) and 31-40 years old (21.1% seasonal and 19.3% pandemic influenza) [5]. This might result from a combination of acquired immunity, vaccination

| Characteristic | Frequencies | | |
	Seasonal (n=149)[a]	Pandemic (n=88)[a]	*p* Seasonal/Pandemic
Cardiopathy			
Yes	16 (13.7%)	6 (7.5%)	p=0.177
No	101 (86.3%)	74 (92.5%)	
Pneumopathy			
Yes	14 (11.2%)	11 (13.6%)	p=0.737
No	103 (88.8%)	70 (86.4%)	
Renal			
Yes	2 (1.6%)	0	p=0.238
No	114 (91.9%)	80 (100%)	
Immunosuppression			
Yes	4 (3.4%)	5 (6.2%)	p=0.350
No	113 (96.6%)	75 (93.8%)	
Smoking habit			
Yes	12 (10.2%)	8 (10.0%)	p=0.969
No	106 (89.8%)	72 (90.0%)	
Metabolic disease			
Yes	8 (6.7%)	2 (2.5%)	p=0.181
No	111 (93.3%)	78 (97.5%)	
Hemoglobinopathy			
Yes	3 (2.6%)	0 (0%)	p=0.149
No	114 (97.4%)	80 (100%)	
Death			
Yes	0	11 (12.9%)	*p=0.001
No	120 (100%)	74 (87.1%)	

[a]Not all characteristics were available for all patients
*Statistically significant by Chi-Square Test

Table 3: Frequencies of clinical conditions and disease evolution in patients infected by pandemic and seasonal influenza A virus.

and also a public health program focused on educative information such as hand washing and other preventive behaviors. Infections leading to death were observed only in patients infected with the pandemic virus (12.9%); this frequency was higher when compared with the 2009 pandemic period when 5.9% of patients infected with A(H1N1)pdm09 died. This result suggests that the pandemic virus could have acquired additional pathogenicity, overcoming the host's immunity. On the other hand, considering the whole population presenting SARI, the mortality rate (5.3%) was similar to that observed in the pandemic period.

The fact that no death cases were observed among patients infected by seasonal influenza A suggests that the public health policies were considerably efficient and the patient management was more appropriate in the post-pandemic period, including extensive antiviral treatment and prophylaxis. Nevertheless, 79% of the patients that were infected by influenza A virus did not receive vaccine, a fact that reveals a gap in the vaccination program in that year.

Further studies are needed in order to infer possible molecular changes in the viral genome that could be associated with differences in its pathogenicity during the pandemic and the post- pandemic periods. A study that analyzed samples from a different region of Brazil aiming the monitoring of antiviral resistance to neuraminidase inhibitors showed that the prevalence of mutants did not support the detection of resistance strains of A(H1N1)pdm09 virus during 2009-2010 [8]. Another study that analyzed only samples from RS found a low prevalence of the mutations H275Y and S247N of the NA protein in strains circulating between 2009-2011 [9]. In a recent study with Brazilian clinical samples, Ferreira et al. showed a significant association between the D239G substitutions in the haemagglutinin (HA) gene of pandemic influenza A H1N1 virus with mortality [10]. In the present study, seasonal influenza A virus was detected co- circulating with the pandemic strain throughout the whole year of 2011, corroborating other studies that suggest that influenza genetic diversity is generated continually in tropical regions [11]. The next step of this study will be to evaluate how viral genome alterations may be associated with drug resistance and factors of virulence.

During the post-pandemic period, viremia was higher in patients infected by seasonal influenza A, as opposed to the pandemic period, when viral loads were higher in patients infected by the pandemic virus and is in accordance with other study realized in China with samples collected out of the pandemic period [5,12]. Even though vaccination could contribute to lower viremia, most of the patients with pandemic virus infection (90.5%) were not vaccinated; regardless of they displayed low viremia, a high rate of mortality was observed among these patients (12.9%).

In this present study were analyzed samples from patients presenting acute respiratory infection symptoms during the post-pandemic period. Therefore, most patients presented fever, cough, dyspnea, myalgia and rhinorrhea; conjunctivitis was the least frequent symptom. Considering all the mentioned symptoms, fever, dyspnea and conjunctivitis showed a positive correlation with infection by A(H1N1) pdm09 virus. Unlike the pandemic period, no significant association was found between these symptoms and viremia [5]; in contrast, when other clinical conditions are taken into account, a positive association was found between high viral loads and immunosuppression as well as metabolic disease in patients infected by seasonal influenza A. Lee et al. [13] report a correlation between viral loads and systemic

comorbidities emphasizing that the use of systemic corticosteroids to treat concomitant medical conditions during influenza infection is associated with a slow decrease in viremia. These can explain the association between immunosuppression and higher viral load within the seasonal group. Yet these data should be interpreted cautiously due to the varied quality of data regarding underlying diseases and small numbers of complete *Influenza Notification Forms*.

As a matter of fact, one of the limitations of this study was that the *Influenza Notification Form* was not uniformly filled out by the different health units' clinician/nurse. Therefore, some demographic and clinical data were missing, reducing our sample number – of 107 samples positive for pandemic influenza A and 182 samples positive for seasonal influenza A samples, only 88 and 149 had complete forms for analyses, respectively, which means a loss of approximately 18%. Finally, vaccination status was also not informed for all patients and this information is of major interest to evaluate the vaccine's efficacy and for public health measures.

In summary, the data demonstrated marked differences among pandemic and post-pandemic periods about dynamics of host, environment and pathogens. Molecular characteristics such as virus' genome sequencing should be considered in future strategies to understand viral pathogenesis. Brazilian surveillance has been effective to implement policies of outbreaks contention through vaccination programs and antiviral treatment offered by the public health system. These strategies include improvement of laboratory capacity, information about prevention, availability of prophylaxis treatment and global molecular studies.

Grant Sponsors

Conflict of Interest

The authors of this study declare they do not have any kind of conflict of interest.

References

1. WHO (2013) Pandemic H1N1 2009 - update 181. Global Alert a Response (GAR/Disease Outbreak News/Weekly Update.

2. Choppin PW, Scheid A (1980) The role of viral glycoproteins in adsorption, penetration, and pathogenicity of viruses. Rev Infect Dis 2: 40-61.

3. Ferguson NM, Galvani AP, Bush RM (2003) Ecological and immunological determinants of influenza evolution. Nature 422: 428-433.

4. Gregianini TS, Dambrós BP, Tumioto GL, Seadi C, Baccin TG, et al. (2011) Diagnóstico Laboratorial da Influenza no Rio Grande do Sul, 2009 a 2011. Boletim da Saúde 13: 13-16.

5. Gorini da Veiga AB, Kretzmann NA, Corrêa LT, Goshiyama AM, Baccin T, et al. (2012) Viral load and epidemiological profile of patients infected by pandemic influenza a (H1N1) 2009 and seasonal influenza a virus in Southern Brazil. J Med Virol 84: 371-379.

6. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 25: 402-408.

7. Dapat IC, Dapat C, Baranovich T, Suzuki Y, Kondo H, et al. (2012) Genetic characterization of human influenza viruses in the pandemic (2009-2010) and post-pandemic (2010-2011) periods in Japan. PLoS One 7: e36455.

8. Souza TM, Mesquita M, Resende P, Machado V, Gregianini TS, et al. (2011) Antiviral resistance surveillance for influenza A virus in Brazil: investigation on 2009 pandemic influenza A (H1N1) resistance to oseltamivir. Diagn Microbiol Infect Dis 71: 98-99.

9. Marx C, Gregianini TS, Lehmann FK, Lunge VR, Carli Sd, et al. (2013)

Oseltamivir-resistant influenza A(H1N1)pdm09 virus in southern Brazil. Mem Inst Oswaldo Cruz 108.

10. Ferreira JL, Borborema SE, Brígido LF, Oliveira MI, Paiva TM, et al. (2011) Sequence analysis of the 2009 pandemic influenza A H1N1 virus haemagglutinin gene from 2009-2010 Brazilian clinical samples. Mem Inst Oswaldo Cruz 106: 613-616.

11. Russell CA, Jones TC, Barr IG, Cox NJ, Garten RJ, et al. (2008) The global circulation of seasonal influenza A (H3N2) viruses. Science 320: 340-346.

12. To KK, Chan KH, Li IW, Tsang TY, Tse H, et al. (2010) Viral load in patients infected with pandemic H1N1 2009 influenza A virus. J Med Virol 82: 1-7.

13. Lee N, Chan PK, Hui DS, Rainer TH, Wong E, et al. (2009) Viral loads and duration of viral shedding in adult patients hospitalized with influenza. J Infect Dis 200: 492-500.