DYNAMICS OF A/H1N1 PANDEMIC VIRUS INFECTION IN ROMANIA – THERAPEUTIC IMPLICATIONS

A. Streinu-Cercel¹, Adriana Pistol², Rodica Bacruban¹, D. Oțeleana¹, Daniela Pițigoi¹, Anca Streinu-Cercel¹, C. Apostolescu¹, Arina Bâlăița¹, Doina Iovânescu¹, Amalia Canton³, Fl. Popovici¹ Simona Paraschiv³

¹ National Institute for Infectious Diseases “Prof. Dr. Matei Balș” Bucharest
² National Centre for Surveillance and Control of Transmissible Disease
³ Ministry of Health

Abstract. A comparative analysis of the 3 types of influenza viruses – pandemic, seasonal and avian (H5N1) – revealed considerable discrepancies in defining risk groups, types of clinical onset and attack and lethality rates. Processing the lessons learned from past influenza epidemics and from the H5N1 infection, the medical world developed action blueprints for the eventuality of pandemic focused on reducing the death rate calculated according to the lethality index. A continual calculation of the fatality rate lead to noteworthy changes in the management of the A/H1N1v infected case, changes regarding the decision to hospitalize and the length of the specific therapy. A strict surveillance of the clinical cases and of the respective contacts substantially contributed to limiting the circulation of this new A/H1N1v virus over the summer and over the beginning of autumn. Epidemiological and clinical evaluation of the viral dynamics is crucial to the management of the current pandemic.

Keywords: A/H1N1v flu virus, pandemic, therapeutics, epidemiology, avian flu

Introduction

The past years have provided data on influenza viruses and the risk of an occurring pandemic with a virus such as this particular new influenza strain. The identification of the avian flu virus circulations’ onset, generating a series of human infections, determined the WHO to consider the possibility of transformation of this particular virus into an eventual pandemic flu virus. These were the grounds for a series of recommendations which engendered the preparation and elaboration of the national plans for fighting against an eventual flu virus pandemic.

Lessons learned from the infections with seasonal flu viruses and with the avian flu virus

Influenza A is a significant threat to society due to the fact that it has the potential of infecting 30% of the world’s population in only a few months, resulting in ~135 million deaths in the first pandemic year, at a conservative overall mortality rate of 2%. This figure represents four times the number of deaths due to HIV-1 over the past 30 years¹.
Frequent RNA copying errors result in high mutation rates and in the emergence of viral variants. In this context, RNA viruses such as influenza A generate errors when copying their RNA, errors resulting in a high mutation rate\textsuperscript{2,3}. An average of 1 mutation is generated per replication per genome for Influenza A\textsuperscript{3}. This error rate in replication is \textasciitilde 300 times higher than the corresponding rate in DNA viruses\textsuperscript{2}. This is because the RNA virus polymerases cannot ‘proof-read’\textsuperscript{2,3}. Most mutations are deleterious but some are beneficial\textsuperscript{2,3}, which explains the enormous adaptive capacity of flu\textsuperscript{2}.

The human immune response to flu is mostly directed towards two surface proteins (haemagglutinin and neuraminidase)\textsuperscript{1}. Changes in these proteins lead to epidemics or pandemics.

Influenza antigenic ‘drift’ and ‘shift’ facilitate immune evasion, also resulting in epidemics and pandemics.

Antigenic drift (figure 1) can lead to epidemic flu through accumulation of point mutations in the antigen-binding sites of haemagglutinin (H) and/or neuraminidase (N). Certain host antibodies (Ab) are unable to recognize/bind to mutant H. Thus, the flu virus spreads more easily through partially immune human (host) population.

![Point mutations in H](image1)

![Novel H-subtype acquired by virus in bird or pig host](image2)

Particular to influenza A viruses is the fact that antigenic shift (figure 2) can lead to pandemic flu\textsuperscript{4}. As genetic recombination/re-assortment occurs, H (sometimes N) is replaced by a novel subtype (when the virus enters a bird/pig or due to co-infection with a seasonal and a bird/pig flu). The new variant virus easily spreads through the non-immune human population.

The epidemiologic evaluation of the seasonal flu (table I) shows that the flu virus strains are generally represented by H1N1 and H3N2\textsuperscript{5} with an attack rate of approximately 5 - 15\%\textsuperscript{6,7}.

<table>
<thead>
<tr>
<th>Causative influenza types</th>
<th>Mainly influenza A &amp; B (sometimes C)\textsuperscript{3}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transmission</td>
<td>Human-to-human by aerosols (coughing &amp; sneezing) or touching virus contaminated surfaces</td>
</tr>
<tr>
<td>Currently circulating strains</td>
<td>H1N1 and H3N2\textsuperscript{5}</td>
</tr>
<tr>
<td>Peak incidence</td>
<td>During autumn and winter in temperate regions\textsuperscript{5}</td>
</tr>
<tr>
<td>Attack rate (%)\textsuperscript{*}</td>
<td>5 – 15%\textsuperscript{6,7}</td>
</tr>
<tr>
<td>Total no. of severe cases</td>
<td>3 – 5 million\textsuperscript{1}</td>
</tr>
<tr>
<td>Total no. of deaths worldwide</td>
<td>250,000 – 500,000\textsuperscript{5}</td>
</tr>
<tr>
<td>Estimated Death rate [EU]</td>
<td>8 – 44 deaths per 100,000; 40,000 – 200,000 total deaths</td>
</tr>
<tr>
<td>High-risk groups</td>
<td>Children &lt;2yrs; elderly (&gt;65 yrs); people with underlying chronic medical condition (heart, lung or kidney disease; diabetes)\textsuperscript{5,7}</td>
</tr>
</tbody>
</table>

\textsuperscript{*}The attack rate is the proportion of the population affected by seasonal influenza

---

**Table I. Epidemiology of seasonal flu**

344

*Therapeutics, Pharmacology and Clinical Toxicology*
The peak incidence of influenza-like infections is – for the temperate climate areas – during the cold season; this generates a heightened number of cases over December and January, cases with implications on intensive care assistance. It is therefore estimated that in the EU, the number of deaths due to seasonal flu ranges between 8 - 44 in 100 000 inhabitants, the majority of cases occurring in elderly people (over 65 years of age)\(^5\).

The history of influenza A pandemics (table II) shows that the number of deaths may vary according to the viral strain and that the attack rates for pandemic flu can be as high as 25–50%, from 1-2 million to 20 - 50 million\(^1, 8-10, 11\).

<table>
<thead>
<tr>
<th>Year</th>
<th>Estimated Deaths</th>
<th>Subtype involved</th>
</tr>
</thead>
<tbody>
<tr>
<td>1889–90</td>
<td>1 million</td>
<td>H2N or H3?</td>
</tr>
<tr>
<td>1918–20</td>
<td>20–50+ million</td>
<td>H1N1</td>
</tr>
<tr>
<td>1957–58</td>
<td>1.5–5 million</td>
<td>H2N2</td>
</tr>
<tr>
<td>1968–69</td>
<td>1–2 million</td>
<td>H3N2</td>
</tr>
</tbody>
</table>

Table II. History of influenza A pandemics

Standing out from the seasonal flu viruses, the H5N1 infection in humans displays a series of particular elements such as:

- the main risk factor is contact with poultry
- the rates of H5N1 infection may be decreasing:
  - no. of outbreaks worldwide\(^6\):
    - 4 in 2003
    - peak of 115 cases in 2006
    - 47 by September 24, 2009
- it can be rapidly fatal with atypical symptoms:
  - a boy had diarrhoea & rapid coma\(^2\)
  - no respiratory/flu-like symptoms\(^2\)
- very low attack rate due to poor human-to-human transmission\(^1\).

What has H5N1 (figure 3) taught us?

- H5N1 may be asymptomatic or it may manifest as a mild influenza-like illness\(^20\)
- However, most patients rapidly progress to develop pneumonia (ARDS) and multi-organ failure\(^20,21\)
- Upper respiratory tract symptoms are less prominent with H5N1 infection than with seasonal flu\(^20\)
- Individuals coming into close contact with infected or contaminated poultry are at high-risk of H5N1 infection (e.g. poultry workers)

Figure 3. H5N1 infection in humans
Nonetheless, the episodes of H5N1 infection have registered a median death rate of 59% (figure 3) with a variability ranging between 30 - 100%. The comparative analysis of these deaths leads to the conclusion that the death rate is directly correlated with the sanitary system’s capacity for mechanic ventilation and implicitly with the access to the sanitary system. Also, data on the incubation period, replication site and spread pace have been extremely useful in elaborating the antipandemic plan’s design.

By processing the lessons learned from the H5N1 infection, the medical world focused on developing action blueprints for the eventuality of pandemic, patterns which would reduce as much as possible the death rate, calculated according to the lethality index and which would allow a dynamic approach of the pandemic process (table 3). The prepandemic phases dealt with the careful monitoring of the flu viruses’ circulation, both in the Northern and in the Southern Hemisphere. The isolation of the A/H1N1 flu virus (also known as A/H1N1v in order to differentiate it from the seasonal A/H1N1 virus) and the monitoring of the infections’ expansion lead, on June 11 2009, to WHO declaring phase 6 pandemic alert (table III).

We therefore proceeded to thorough monitoring of the A/H1N1v infections (figure 4) periodically calculating the attack and fatality rates. A prominently increasing trend of the infections induced by the new A/H1N1 flu virus can be easily observed (figure 4).

<table>
<thead>
<tr>
<th>2009 Timeline</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>26 April</td>
<td>H1N1/09 pandemic first reported by US &amp; Mexico; first reported as ‘swine flu’ but origin unknown</td>
</tr>
<tr>
<td>27 April</td>
<td>WHO Phase 4 pandemic alert declared</td>
</tr>
<tr>
<td>29 April</td>
<td>WHO Phase 5 pandemic alert declared</td>
</tr>
<tr>
<td>30 April</td>
<td>57 cases, including 8 deaths reported in 8 countries</td>
</tr>
<tr>
<td>29 May</td>
<td>15,510 cases, including 99 deaths in 53 countries</td>
</tr>
<tr>
<td>11 June</td>
<td>WHO declares Phase 6 pandemic alert</td>
</tr>
<tr>
<td>29 June</td>
<td>70,983 cases, including 311 deaths in 116 countries</td>
</tr>
<tr>
<td>6 July</td>
<td>94,512 cases, including 429 deaths in 135 countries</td>
</tr>
<tr>
<td>11 October</td>
<td>&gt;399,232 confirmed cases and 4,735 deaths</td>
</tr>
<tr>
<td>14 December</td>
<td>&gt;1,000,000 confirmed cases and 10,863 deaths</td>
</tr>
<tr>
<td>Winter 09/10</td>
<td>Fears that H1N1/09 cold become a major global pandemic in winter months</td>
</tr>
</tbody>
</table>

Table III. H1N1/09 pandemic story & timelines

Figure 4. The dynamics of A/H1N1 infections
Source: WHO website. H1N1/09 surveillance. Available at: www.who.int/csr/
The medical world is currently preoccupied with the management of the moderate-severe case of A/H1N1v infection.

A comparative analysis of the 3 types of flu viruses – pandemic, seasonal and avian (H5N1) – shows considerable discrepancies in defining risk groups, types of clinical onset and attack and lethality rates (table IV).

<table>
<thead>
<tr>
<th></th>
<th>Pandemic (H1N1) 2009</th>
<th>Seasonal influenza</th>
<th>Bird flu (H5N1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td>A/California/07/2009</td>
<td>Influenza A (H1N1, H3N2)</td>
<td>H5N1</td>
</tr>
<tr>
<td>Phase of pandemic alert</td>
<td>6</td>
<td>NA</td>
<td>3</td>
</tr>
<tr>
<td>At-risk groups</td>
<td>Underlying medical conditions, young children and adolescents, pregnant, obese</td>
<td>Underlying medical conditions, very young &amp; elderly</td>
<td>Very young, adolescents, young adults</td>
</tr>
<tr>
<td>Transmission</td>
<td>Airborne, human-to-human</td>
<td>Airborne, human-to-human</td>
<td>Contact with infected poultry</td>
</tr>
<tr>
<td>Signs, symptoms</td>
<td>Flu-like symptoms, vomiting, nausea, diarrhoea</td>
<td>Flu-like symptoms</td>
<td>High fever, diarrhoea, vomiting, abdominal pain, chest pain, nose &amp; gum bleeds, severe respiratory disease, multiorgan dysfunction</td>
</tr>
<tr>
<td>Attack rate (%)</td>
<td>20–30 (EU; Aus; NZ)</td>
<td>5–15</td>
<td>Very, very low</td>
</tr>
<tr>
<td>Hospitalization (%)</td>
<td>9</td>
<td>1.2</td>
<td>96</td>
</tr>
<tr>
<td>Mortality (%)</td>
<td>0.1–1.2%</td>
<td>0.1</td>
<td>~60</td>
</tr>
</tbody>
</table>

Table IV. Comparison of pandemic A/H1N1 2009 with seasonal and H5N1 influenza
*Source: WHO website. H1N1/09 surveillance. Available at: www.who.int/csr/*

**Romania’s experience**

Romania’s experience displays 2 different aspects: One of them is related to the circulation of the H5N1 avian flu virus between 2006 - 2007 with particular implications in poultry. The second aspect is the pandemic period per se.

During the first period, we used the collected data for elaborating our own plan for fighting the pandemic, which was then expected to be induced by H5N1.

The pandemic induced by A/H1N1v lead to the involvement of two important networks: an epidemiology network and an infectious diseases network, under the coordination of the Ministry of Health.

Concomitantly, the data regarding the first A/H1N1v epidemic wave were systematically evaluated. This inferred the epidemiologic evidence that the strict surveillance of the clinical cases and of the respective contacts substantially contributed to limiting the circulation of this new virus over the summer and over the beginning of autumn (figure 5). The infections were confirmed through PCR.
Upon calculating the reproduction rate, we managed to evaluate the infection's eventual local dissemination (figure 6). In order to successfully confirm that the infection with A/H1N1 has not spread in the population, the $R$ needs to be maintained <1 \cite{22}.

During May - September, the reproduction rate gravitated around 0.23, confirming a high correlation between the imported cases and the absence of the local inter-human transmission of A/H1N1v (figure 7).

In the given context, the analysis, differentiated on flu virus strains and other acute upper respiratory tract infections (AURTI) + influenza (figure 8) clearly showed a low circulation of the flu viruses compared to that of other viruses with seasonal respiratory tropism. Moreover, after week 25, the incidence
of the infections induced by A/H1N1v started increasing and displaying local transmission. This imposed a reevaluation of the management of cases diagnosed with acute A/H1N1v infection and respectively the implementation of new measures throughout the territory.

Up to October 27 2009, Romania diagnosed and reported 386 cases, out of which 370 cases were validated (96%) – confirmed through PCR.

The analysis of the age groups’ distribution within this first stage revealed the target group of individuals 10 to 34 years old. This steered priority attention to the group of pupils and students (figure 9).

The sex distribution of the cases was: 46% within females and 54% within males (figure 10).
During the first pandemic wave, the majority of cases were imported (figure 11), but also lead to contact cases. The imported cases were 73%, the secondary cases were 26% and the tertiary cases were only 1% of the total cases, which confirmed the fact that the careful monitoring of the princeps cases was vital for keeping the first pandemic wave under control.

Up to week 26, 62 clusters were registered and evaluated: 30 imported and 32 mixed clusters (imported + secondary +/- tertiary cases).

These 62 clusters (table V) included 255 cases out of which 94 were imported and 161 were mixed, with a minimum of 2 cases for both types of clusters and a maximum of 11 cases for the imported clusters and a maximum of 24 cases for the mixed clusters.

<table>
<thead>
<tr>
<th>Cluster type</th>
<th>No. cases</th>
<th>Average</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imported</td>
<td>94</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>11</td>
</tr>
<tr>
<td>Mixed</td>
<td>161</td>
<td>5</td>
<td>3</td>
<td>2</td>
<td>24</td>
</tr>
<tr>
<td>TOTAL</td>
<td>255</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>24</td>
</tr>
</tbody>
</table>

Table V. Clusters’ dimension

Source: National Centre for Surveillance and Control of Transmissible Diseases

The distribution of the sporadic cases within these clusters was of 31,4% (figure 12) vs. 68,6% in cluster.

Figure 11. Distribution of the pandemic A/H1N1 flu cases according to the probable manner of infection (N = 372)

Source: National Centre for Surveillance and Control of Transmissible Diseases

Figure 12. Distribution of the sporadic/cluster cases of pandemic A/H1N1 flu (N=372)

Source: National Centre for Surveillance and Control of Transmissible Diseases
The analysis of the concordance with the case definition and of the complications and need for hospitalization and deaths showed that 54% of the cases corresponded to the case definition (fever and acute respiratory infection signs and symptoms), 72% included history of fever. 17% of the patients displayed primary interstitial pneumonia due to flu, 1% presented secondary bacterial pneumonia and 8% displayed clinical signs of pneumonia. In this context, 68% of the patients required hospitalization and 32% were placed under home isolation. No deaths were registered during this period.

Another analysis targeted the distribution of the symptomatic and asymptomatic cases (figure 13), analysis which showed that practically 96% of the cases were symptomatic whereas only 4% were asymptomatic.

This data aided in estimating the moment when the switch between dominant imported cases versus dominant cases of local transmission.

For 97 secondary and tertiary cases (out of which 90 with known onset date and 75 with probable date of exposure), the incubation (in days) was of:
- average = 4
- median = 3
- min = 1
- max = 10

The analysis of the type of transmission reported to presence or absence of close contact (figure 14) revealed the fact that the main origins of the infecting contact were collectivities (47%), closely followed by families (36%), relations (15%) while the airplane transmission risk was of only 2%.

An important element in preparing for a second pandemic wave was the evaluation of the latencies of detection, confirmation and treatment initiation (table VI). The element playing a pivotal role was the
latency in therapy initiation (a median latency of 3 days). This required a series of subsequent analyses for defining a case management pattern with a therapeutic latency of 24 hours.

<table>
<thead>
<tr>
<th>Latency</th>
<th>Average/median (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Detection (date of onset – date of first medical presentation)</td>
<td>2</td>
</tr>
<tr>
<td>Confirmation (date of onset – date of IC laboratory confirmation)</td>
<td>3</td>
</tr>
<tr>
<td>Treatment initiation (date of onset – date of treatment initiation)</td>
<td>3</td>
</tr>
</tbody>
</table>

Table VI. Evaluation of detection, confirmation and treatment initiation latencies

Source: National Centre for Surveillance and Control of Transmissible Diseases

The analysis of the patient history and predisposing conditions in these patients showed that 11% of the patients had undergone seasonal flu vaccination, 1% had undergone anti-pneumococcal vaccination, 0.3% had received oseltamivir prophylaxis (7 cases – administered prior to laboratory confirmation, but after onset). Predisposing conditions were present in 6.2% of the patients, as follows: pulmonary diseases in 2.4%; of patients, pulmonary asthma in 2.2%, cardiovascular diseases in 2.7%, obesity in 1.6%, diabetes in 0.8% of the patients.

20% of the cases experienced onset abroad, which represents one explanation for the late detection.

All these patients received oseltamivir (98%) or zanamivir (2%) therapy as follows: 49% for 7 days, 24% for 5 days, 15% for 6 days with a maximum of 21 days – in severely immunodepressed patients.

Only 4% of those treated presented moderate adverse reactions: nausea, vomiting, dizziness, minor hepatic cytolytic syndrome and only one case of altered clinical state, nausea, laryngeal spasm and perioral paresthesias.

We concomitantly evaluated the responsiveness to therapy, which translated into conversion to negative RT-PCR. This study enrolled 152 patients out of a total 1770 presented patients (table VII). Out of the 1770 patients presenting for so-called A/H1N1v infections, 897 were evaluated and out of these, 152 were confirmed A/H1N1 cases.

<table>
<thead>
<tr>
<th>No. of cases</th>
<th>Absolute value</th>
<th>% out of presented cases</th>
<th>% out of evaluated cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presented cases</td>
<td>1770</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Evaluated cases</td>
<td>897</td>
<td>50</td>
<td>100</td>
</tr>
<tr>
<td>Positive</td>
<td>152</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Negative</td>
<td>745</td>
<td>42</td>
<td>83</td>
</tr>
</tbody>
</table>

Table VII. Rate of A/H1N1v positivity out of total presenting cases during May - August 2009 in the National Institute for Infectious Diseases „Prof. Dr. Matei Balș”

Source: National Institute for Infectious Diseases „Prof. Dr. Matei Balș” Bucharest

The dynamics of the presentations to the emergency room of the National Institute for Infectious Diseases “Prof. Dr. Matei Balș” Bucharest was conditioned by TV messages on the one hand and by broadcasts of foreign media and announcements posted in international airports on the other hand. Hence, people started to become preoccupied with their health state and with an eventual infection with A/H1N1v flu virus. This phenomenon occurred both in adults (figure 15) and in children (figure 16).

Whereas in adults the females registered a higher incidence, the opposite was noticed in children, the sex distribution displaying a higher incidence of presentations in boys.
The A/H1N1v positivation rate constantly increased over May - August, with a global predominance in men (figure 17). Also, most cases were imported (figure 18), the local transmission being limited to immediate contacts.

Figure 15. Presentation dynamics – adults
Source: National Institute for Infectious Diseases „Prof. Dr. Matei Balș“ Bucharest

Figure 16. Presentation dynamics – children
Source: National Institute for Infectious Diseases „Prof. Dr. Matei Balș“ Bucharest

Figure 17. Dynamics of positive cases per month
Source: National Institute for Infectious Diseases „Prof. Dr. Matei Balș“ Bucharest
The symptomatology was governed by pharyngitis, headache and coryza, which called for an extension of the case definition (figure 19), so as to include myalgia – in 82% of patients (figure 20).

In all 152 patients, the presence of viral RNA was determined through RT-PCR up to the conversion to negative RT-PCR. The determinations were performed at moment zero, at 3 days, 5 days, 7 days, 10 days, 14 days and 21 days (figure 21).

This investigation showed that after 7 days of treatment, only 75% of the patients were negative for viral RNA (21% after 5 days and 54% after 7 days of treatment). After 10 days of treatment, 85% of the patients had eliminated the virus, with conversion to negative RT-PCR.
In severely immunodepressed patients the conversion to negative viral RNA – under therapy – was obtained after 21 days (2%).

This denotes the fact that in the severe (critical) patient, the decision of discontinuing the specific antiviral therapy must be made according to the negative conversion of the viral RNA – demonstrated by RT-PCR.

![Figure 21. PCR undetectability according to median number of therapy days](Source: National Institute for Infectious Diseases „Prof. Dr. Matei Balș” Bucharest)

In line with this data, the lethality index was computed (figure 22). The continual calculation of the fatality rate lead to noteworthy changes in the management of the A/H1N1v infected case, changes regarding the decision to hospitalize and the length of the specific therapy.

![Figure 22. Fatality index](Source: Ministry of Health)

**Discussions**

The dynamics of the pandemic process of A/H1N1v compared to the seasonal flu viruses and to the H5N1 avian flu virus clearly shows a series of important differences both in the type of clinical outline and in the risk of death by infection.

What H5N1 has taught us has determined us to be more vigilant in the evaluation of the viral dynamics both epidemiologically and clinically. It is epidemiologically clear that therapy with oseltamivir does not reduce the risk of contamination, but it does genuinely modify the clinical symptomatology when its administration is begun early – during the first 24 hours from clinical onset.

In immunodepressed patients, oseltamivir therapy must be continued up to proof of conversion to negative viral RNA and it must not be limited to the “on label” indication of the compound.
References

5. WHO seasonal flu fact sheet No 211. Available at: http://www.who.int/mediacentre/factsheets/fs211/en/