Predictors of Pandemic (H1N1) 2009 Virus Positivity and Adverse Outcomes among Hospitalized Patients with a Compatible Syndrome

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ABSTRACT: **Background:** A pandemic (H1N1) influenza A virus was identified in 2009.

Objectives: To investigate predictors for pandemic (H1N1) 2009 virus infection among hospitalized patients with a flu-like illness and to identify parameters suggesting a severe clinical course.

Methods: We analyzed a cohort of all patients hospitalized during a 2 month period with a flu-like syndrome who were tested for pandemic (H1N1) 2009 infection. Demographic, clinical and laboratory, along with outcome parameters, were recorded and compared between pandemic (H1N1) 2009 virus-positive and negative hospitalized patients.

Results: Of the 179 examined hospitalized patients suspected of having pandemic (H1N1) 2009 infection 65 (36%) were found positive. These patients tended to be younger and had significantly fewer comorbidities. In addition, they had a significantly higher frequency of fever (94%), cough (86%) and myalgia (29%). Furthermore, age < 65 years and cough were independent predictors for pandemic (H1N1) 2009 virus positivity in a multivariate regression analysis. Notably, 14 of the 65 positive patients (21.5%) had acute respiratory insufficiency requiring treatment in the intensive care unit. These patients were neither older nor previously sicker than patients with non-severe disease, but were distinguished by augmented inflammatory markers, significant lymphopenia associated with disease severity, and overall mortality of 21.4%.

Conclusions: Pandemic (H1N1) 2009 virus-positive hospitalized patients tend to be younger and have fewer comorbidities as compared to compatible negative patients. A significant number of relatively young and previously healthy positive patients might develop severe disease associated with a robust inflammatory reaction and significant lymphopenia.

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KEY WORDS: pandemic (H1N1) 2009 virus, prognosis, mortality, intensive care unit

In late March 2009, an outbreak of respiratory illness occurred in Mexico that was later identified as a novel pandemic (H1N1) 2009 virus-related disease [1,2]. The first reports from Mexico of severe illness accompanied by respiratory failure and death among young previously healthy people infected with this virus suggested that this infection is a potentially serious health concern [3,4]. Accordingly, reports about the rapid spread of the virus to many countries outside of Mexico brought the World Health Organization to classify the global spread of the virus as an international concern with a pandemic level of six [5].

The lack of adequate data on the natural history of pandemic (H1N1) 2009 virus infection outside of Mexico led to uncertainty in approach to this new pandemic in the medical communities of many countries around the world. Furthermore, the first reports outside of Mexico of an unusual susceptibility of younger and previously healthy people to severe respiratory complications due to this virus [6-8] led to an ambivalence in approach to young patients with signs and symptoms of influenza [9]. Therefore, one of the major challenges in the combat with the H1N1 pandemic today is to identify patients presenting with flu-like illness who are more likely to be infected with this virus, and to identify the parameters predicting severe morbidity and mortality.

We present a case series of all consecutive patients hospitalized in a major medical center in Israel during a 2 month period with a presumed diagnosis of pandemic (H1N1) 2009 virus infection. Demographic, clinical and laboratory, along with outcome parameters, were analyzed in order to identify possible factors that may suggest the diagnosis of pandemic (H1N1) 2009 virus infection among patients with a flu-like illness, and furthermore, to examine which parameters can predict a more severe clinical course among these hospitalized patients.

PATIENTS AND METHODS

This study was conducted in the Tel Aviv Sourasky Medical Center, the major community and tertiary care university

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hospital in Tel Aviv (1150 beds), serving a population of approximately 750,000 people. The study population comprised all consecutive patients (n=179), including children, who were hospitalized during the 2 month period of the analysis (July–August 2009) and tested for the presence of pandemic (H1N1) 2009 infection based on clinical suspicion. The decision to hospitalize suspected patients was based on comorbidity and symptom severity, hypoxia or radiographic evidence of pneumonia. The data of the admitted 179 patients were collected and analyzed.

Treatment with oseltamivir was initiated in virtually all hospitalized patients with suspected pandemic (H1N1) 2009 virus infection. Treatment was stopped upon validation of the patient's negativity for this infection based on the reverse transcriptase-polymerase chain reaction test.

DATA COLLECTION AND STUDY DESIGN

Demographic, clinical and laboratory data were obtained by retrospectively reviewing patients' medical records. The study was designed as a case series of all the 179 patients tested for pandemic (H1N1) 2009 infection, with subsequent comparison between the virus-positive and negative groups, and further analyzing the positive patients for difference between those with and without a severe clinical course. The study was approved by the hospital's institutional review board. Comorbidities were rated according to the Charlson index, a validated prognostic comorbidity score that takes into account the number and severity of comorbid diseases, with higher scores representing more severe comorbidities [10].

PANDEMIC (H1N1) 2009 VIRUS TEST

Nasal and throat viral swabs (a total of three) were obtained from suspected patients and were transferred refrigerated to the Israeli reference laboratory for RT-PCR testing according to WHO recommendations.

STATISTICAL ANALYSIS

Analyses, using Stata version 9 (Stata Corp., College Station, TX, USA), were performed with respect to the main study aim: predicting H1N1 positivity among hospitalized tested patients. Student's *t*-test was used to compare normally distributed continuous variables, Wilcoxon rank sum test for non-normally distributed variables. Chi-square test was used to analyze dichotomized variables. Multivariable analysis was performed using logistic regression. All variables with a P value < 0.1 were included in the models, and those with $P \leq 0.05$ were retained in the model. Area under the ROC curve was calculated to estimate the model's ability to predict positivity. $P \leq 0.05$ was considered statistically significant.

RT-PCR = reverse transcriptase-polymerase chain reaction
WHO = World Health Organization

RESULTS

DEMOGRAPHIC, CLINICAL AND LABORATORY PARAMETERS

During the 2 month period July to August 2009, 179 patients admitted to our hospital were tested for the presence of S-OIV (swine-originated influenza virus), based on the presence of signs and symptoms of upper respiratory tract infection. Treatment with oseltamivir was initiated in almost all patients, but only 65 of the 179 (36%) were ultimately found to be positive for pandemic (H1N1) 2009 infection.

As shown in Table 1, H1N1 virus-positive patients tended to be younger with significantly fewer comorbidities, as compared to negative patients. In addition, typical signs and symptoms of flu-like illness such as fever, cough and myalgia were significantly more common among H1N1-positive patients [Table 1].

Table 1. Demographic, clinical and laboratory characteristics of admitted patients tested for the presence of pandemic (H1N1) 2009 virus infection

Variable	S-OIV positive (N=65)	S-OIV negative (N=114)	*P* value
Gender (%, Male/Female)	55.4/44.6	57.9/42.1	0.74
Age (yrs, mean ± SD)	37 ± 21.7	44.9 ± 29.8	0.06
Comorbidity score (mean ± SD)*	1.26 ± 1.72	2.38 ± 2.45	0.001
Time to S-OIV sample**	1.31 ± 3.2	2.71 ± 5	0.045
Samples on first day (%)	76.9	57	0.007
Symptoms [No. of patients (%)]			
Fever	61 (93.8)	86 (75.4)	0.002
Cough	56 (86.2)	54 (47.4)	< 0.0001
Dyspnea	22 (33.9)	47 (41.2)	0.33
Myalgia	19 (29.2)	17 (14.9)	0.021
Sore throat	12 (18.5)	16 (14)	0.4
Abdominal pain	2 (3.1)	10 (8.8)	0.14
Vomiting	15 (23.1)	18 (15.8)	0.22
Diarrhea	12 (18.5)	12 (10.5)	0.13
Fever+cough+myalgia	16 (24.6)	12 (10.5)	0.013
Laboratory values [Median (IQR*)]			
BUN_{max} (mg/dl)	14 (10-24)	18 (11-40)	0.06
LDH_{max}(IU/L)	501 (360-792)	538 (399-865)	0.19
ALT_{max}(U/L)	32 (23-67)	33 (21-76)	0.9
CK_{max}(IU/L)	131 (74-654)	94 (65-287)	0.019
$Lymphocytes_{min}$ ($x10^3/\mu l$)	0.8 (0.5-1.32)	0.8 (0.4-1.4)	0.74
WBC_{max} ($x10^3/\mu l$)	9.4 (7.15-13.7)	9.8 (8.1-18.2)	0.14
$Platelets_{max}$ ($x10^3/\mu l$)	269 (225-422)	319 (242-508)	0.09
CRP_{max} (mg/dl)	58 (30-153)	65 (20-149)	0.9

* Charlson comorbidity score was calculated as described in Methods.

** Days from admission

S-OIV = swine-originated influenza virus, IQR = interquartile range, CRP = C-reactive protein, LDH = lactate dehydrogenase, ALT = alanine aminotransferase, CK = creatine kinase.

However, no significant difference in major laboratory values, except for a slight rise in the level of creatine kinase was detected between the virus-positive and negative patients [Table 1].

Multivariable logistic regression identified two variables as important independent predictors of H1N1 positivity: age younger than 65 (odds ratio 12.5, $P < 0.0001$) and the presence of cough (OR 5.9, $P < 0.0001$). The model had an area under the ROC curve of 0.81, suggesting a very good prediction.

Interestingly, the mean time from admission to the first sampling for the presence of pandemic (H1N1) 2009 virus was significantly shorter among the virus-positive patients, reflecting the much higher percentage of patients among the positive patients for whom a H1N1 virus sample was taken on admission: 77% in the virus-positive group versus 57% in the negative group.

OR = odds ratio

Table 2. Comparison of clinical outcome parameters during hospitalization between pandemic (H1N1) 2009 virus-positive and negative patients

Variable	S-OIV positive (n=65)	S-OIV negative (n=114)	*P* value
Duration of hospitalization in patients suspected on admission (days, mean ± SD)	3 ± 2.5	4.1 ± 3.7	0.12
Pneumonia	28 (43.1%)	43 (37.7%)	0.5
Mechanical ventilation	9 (13.8%)	12 (10.5%)	0.5
Transfer to ICU	14 (21.5%)	16 (14%)	0.2
Death	3 (4.6%)	8 (7%)	0.5

Table 3. Comparison of demographic, clinical and laboratory characteristics of ICU vs. non-ICU pandemic (H1N1) 2009 virus-positive patients

Variable	ICU (n=14)	Non-ICU (n=51)	*P* value
Demographic			
Age (yrs, mean ± SD)	35.4 ± 23.4	37.5 ± 21.5	0.7
Gender (%, Male/Female)	42.9/57.1	58.8/41.2	0.29
Co-morbidity score (mean ± SD)*	0.86 ± 1.83	1.37 ± 1.68	0.3
Laboratory data [Median (IQR)]			
BUN_{max} (mg/dl)	25 (11-58)	13 (9-18)	0.01
LDH_{max}(IU/L)	833 (607-1096)	404 (305-531)	< 0.0001
ALT_{max}(U/L)	135 (39-178)	28 (21-46)	0.0001
CK_{max}(IU/L)	922 (90-1945)	117 (73-416)	0.027
$Lymphocytes_{min}$ (x10^3/μl)	0.5 (0.2-0.725)	0.9 (0.625-1.47)	0.0029
WBC_{max} (x10^3/μl)	14.9 (10.5-22)	8.8 (6.8-11.5)	0.002
$Platelets_{max}$ (x10^3/μl)	458 (262-724)	250 (207-328)	0.004
CRP_{max} (mg/dl)	165 (105-196)	39 (23-87)	0.0001
Outcome			
Duration of hospitalization in patients suspected on admission (days, mean ± SD)	5.6 ± 2.7	2.6 ± 2.2	0.0026
Pneumonia	14 (100%)	14 (27.5%)	< 0.0001
Mechanical ventilation	9 (64%)	0 (0)	< 0.0001
Death	3 (21)	0 (0)	0.0007

* Charlson comorbidity score was calculated as described in Methods.

CLINICAL OUTCOMES

Reports from Mexico, where the current pandemic originated, suggest that the new pandemic (H1N1) 2009 virus has the potential to cause a severe illness with life-threatening respiratory complications, especially among young previously healthy people [1,2]. To determine whether this is also the case in our study population we analyzed major clinical outcomes of patients who were tested for the presence of the virus, and compared those outcomes between the virus-positive and negative patients. Interestingly, although H1N1 virus-positive patients had fewer comorbidities and tended to be younger than the negative patients [Table 1], the two groups of patients were comparable in the occurrence of pneumonia, a well-known complication of influenza in general and of the pandemic (H1N1) 2009 virus strain in particular [Table 2]. Furthermore, there was no difference in the length of hospital stay or in the rate of transfer to the ICU, mechanical ventilation or death between the two groups of patients, despite the difference in age and background comorbidity, suggesting that pandemic (H1N1) 2009 infection tends to be a relatively serious disease in a significant proportion of hospitalized patients.

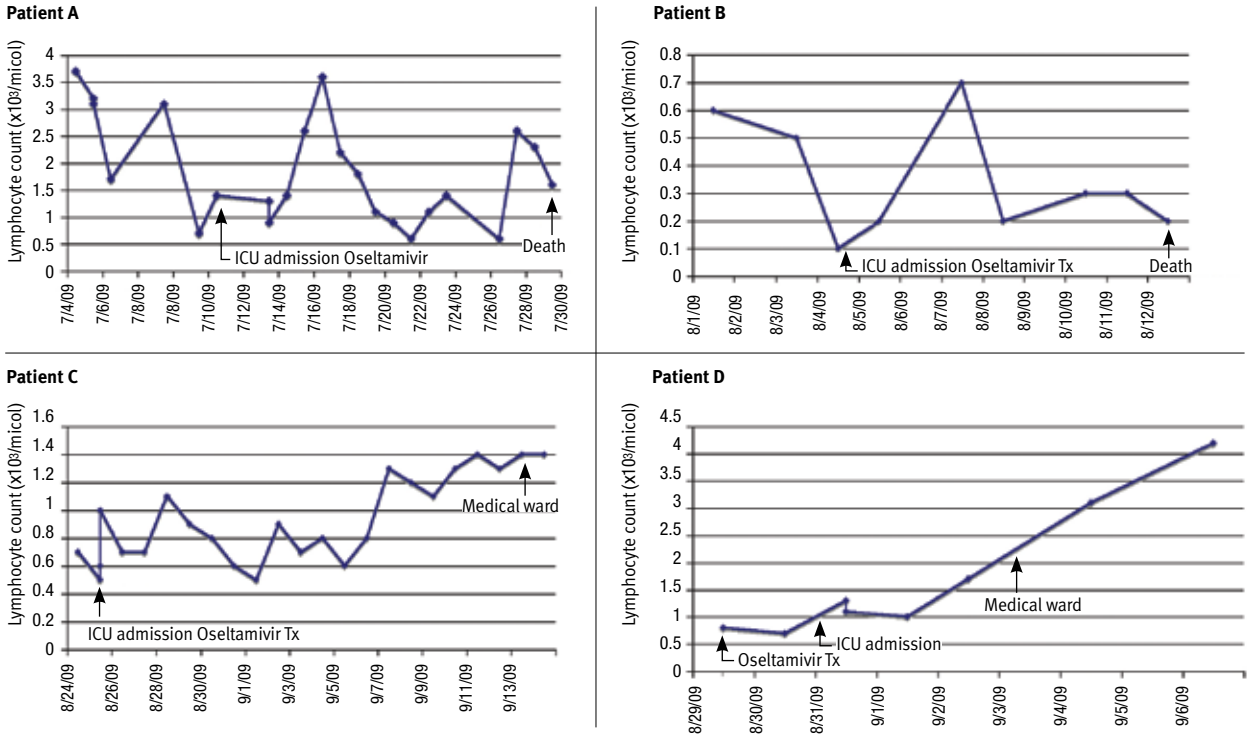
SEVERE DISEASE AND NON-SEVERE DISEASE

Of the 65 H1N1-positive patients 14 (21.5%) were transferred to the ICU during their hospitalization, most of them with respiratory failure that reflected a much more severe clinical course. We analyzed demographic, clinical and laboratory parameters of these patients and compared them to those of the infected patients who experienced a milder clinical course that did not necessitate a transfer to the ICU. As shown in Table 3, H1N1-positive patients with a severe clinical course were neither older nor did they have more comorbidities, as compared to virus-positive patients who experienced a milder disease. However, the complicated cases were distinguished by significantly higher indices of inflammation, such as leukocytosis, thrombocytosis and elevated C-reactive protein, and by much higher blood urea nitrogen, lactate dehydrogenase, alanine aminotransferase and creatine kinase. Noteworthy is the significant severe lymphopenia observed among the ICU patients, compatible with previous reports from Mexico about lymphopenia accompanying severe pandemic (H1N1) 2009 infection [4].

As expected, clinical outcomes were worse among the ICU patients and the length of their hospital stay was significantly longer, with higher rates of pulmonary complications that

ICU = intensive care unit

Figure 1. Lymphopenia among ICU-admitted pandemic (H1N1) 2009 virus-positive patients correlates with disease severity. Graphic presentations of the lymphocyte counts of four representative ICU-admitted virus-positive patients (patients A-D) along their hospital course. Times of oseltamivir initiation, transfer to the ICU, readmission to the medical ward and death are indicated.



necessitated mechanical ventilation. Notably, pulmonary complications were the hallmark of a severe clinical course, reflected by the high percentage (50%) of patients with pulmonary infiltrates who developed respiratory failure and were transferred to the ICU. Consequently, 3 of the 14 ICU patients (21.4%) ultimately succumbed to their disease, whereas no cases of death occurred among the non-ICU pandemic (H1N1) 2009 virus-infected patients [Table 3].

CORRELATION BETWEEN LYMPHOPENIA AND DISEASE PROGRESSION

As noted above, pandemic (H1N1) 2009-infected patients with a severe clinical course, as defined by the need for their transfer to the ICU, were significantly more lymphopenic than non-ICU patients [Table 3]. The correlation between the lymphocyte count and the clinical course of the disease among virus-infected complicated patients is depicted in Figure 1. All four representative patients had significant lymphopenia that was at its nadir level around the time of their transfer to the ICU, correlating with the time of clinical deterioration and signs and symptoms of respiratory failure. Interestingly, initiation of treatment with oseltamivir led to a rise in the lymphocyte counts in all patients. However, whereas the lymphocyte counts of patients C and D continued to increase steadily correlating with the patients' recovery, patients A and B experienced an initial response in terms of lymphocyte counts that was immediately abrogated, associated with clinical deterioration and death. These observations suggest that lymphopenia is associated with a severe clinical course among pandemic (H1N1) 2009 virus-infected patients and its resolution tends to correlate with clinical recovery.

DISCUSSION

The emergent pandemic (H1N1) 2009 virus is challenging to the medical community around the world because of its unusual rapidity of spread and its tendency to complications among relatively young and previously healthy patients, seemingly considered a low risk group for influenza-induced complications [3,4,11]. However, despite increasing epidemiologic reports from countries outside of Mexico to which the virus has spread [6-8], it is still difficult to predict the natural course of the disease and establish strict policies for health care providers. The current study, which is based on a case series of H1N1-infected patients, seems to substantiate current knowledge on the natural history of the disease and also suggests predictors for both pandemic (H1N1) 2009 virus positivity among hospitalized patients with a flu-like syndrome and for a more severe clinical course among infected patients.

Several major conclusions may be drawn from the present analysis. First, among hospitalized patients suspected of having H1N1 infection, those found to be positive for the virus tend to be younger and have significantly fewer comorbidities as compared to those who are negative. Second, our study clearly shows that the typical symptoms and signs of influenza, including fever, cough and myalgia, are more common among pandemic (H1N1) 2009 virus-positive patients. Thus, supported by a multivariable logistic regression analysis of our data, we conclude that younger age and the presence of cough on presentation are two parameters that may predict H1N1 positivity among hospitalized patients with a flu-like illness. Obviously, the younger age of the positive patients can be explained by other medical conditions that can present with respiratory symptoms and are much more prevalent among older patients with comorbidities. Indeed, the observation that the pandemic (H1N1) 2009 virus-negative patients were tested for the presence of the virus later during their hospitalization course as compared to the positive patients reflects the ambiguity in the differential diagnosis of these patients' clinical presentation.

Most importantly, a significant number of infected patients were prone to severe complications and respiratory failure that necessitated an urgent transfer to the intensive care unit. These patients were distinctive for the presence of increased inflammatory markers. This observation may correlate with immune system over-reaction to the viral infection, leading to the so-called cytokine storm, which triggers inflammation and lung damage that can lead to multiple organ failure and death [12,13].

Interestingly, in contrast to the typical seasonal flu that tends to complications in older and debilitated patients [14], the severely ill H1N1-infected patients were neither older nor previously sicker than the H1N1-infected patients with a milder clinical course. This observation joins a growing body of evidence indicating that the novel pandemic (H1N1) 2009 virus tends to complications in relatively young people who were traditionally thought to be at a low risk for flu-related complications [1-4,6-8,11]. Recent evidence suggests that the younger age of the severely infected patients may be due to the presence of cross-reacting protective antibodies found in older individuals who have been exposed to the Spanish flu virus. These antibodies cross-react with the present pandemic (H1N1) 2009 virus, providing the elderly population a relative protection from the present epidemic [15]. However, in contrast to recent studies suggesting a severe clinical course in patients with at least one significant comorbidity [6-8], the patients with a severe clinical course in our study had no more comorbidities than patients with a non-severe clinical course. However, the three cases of deaths had significant comorbidities (one patient after bone marrow transplantation, one with metastatic colon cancer and one with a history of alcohol abuse and intavenous drug use) that apparently led to the fatal outcome.

The present analysis has several limitations, mainly its retrospective nature and its restriction to hospitalized patients. In addition, some of the admitted patients suspected of having pandemic (H1N1) 2009 infection might have been falsely categorized as non-infected due to late sampling. However, the design of the study is advantageous in its focus on a defined group of admitted patients suspected of having the infection, thus enabling reliable validation of demographic, clinical and laboratory data on admission and during the patients' hospital course, as well as their clinical outcomes. Obviously, it should be emphasized that the results of the study are not applicable to the course of pandemic (H1N1) 2009 virus infection in the non-admitted general population, but rather to patients whose clinical presentation was severe enough to justify their admission. In addition, our study was performed during a limited period at the beginning of the pandemic (H1N1) 2009 virus outbreak in Israel (July–August 2009). Therefore, as the characteristics of the pandemic might have changed during the subsequent months, some of our study's conclusions might not be applicable to the situation in 2010.

In conclusion, the data indicate that young and relatively healthy pandemic (H1N1) 2009 virus-infected patients constitute a risk group for a severe clinical course. However, mortality, at least in the present series, was confined to patients with significant concurrent diseases. Interestingly, the data indicate that lymphopenia, sometimes profound, was significantly more common among H1N1-positive patients with severe disease, in accordance with a previous report [3]. Moreover, as shown here, kinetic analysis of the lymphocyte counts among ICU patients during hospitalization suggested a correlation between the level of lymphopenia and severity of the disease. Furthermore, the data also suggest that the absence of recovery in the lymphocyte counts despite oseltamivir therapy might predict a poor outcome among ICU patients. In accordance, a previous study performed in H5N1-infected mice showed that infection with the lethal strains of the virus correlated with a profound drop in the lymphocyte counts following their apoptosis, accompanied by diminished synthesis of cytokines such as interleukin-1 and interferon-gamma [13]. This suggests that certain pathogenic strains of influenza virus might severely harm the immune system, resulting in disseminated and lethal disease, a mechanism that might explain the present observations. Therefore, we suggest that lymphopenia and its kinetics during hospitalization in severely ill H1N1-infected patients might serve as a surrogate marker for the severity of the disease and its prognosis.

In summary, our study shows that both age < 65 and the presence of cough are independent predictors for pandemic (H1N1) 2009 virus positivity among clinically suspected

patients. Notably, in contrast to the typical seasonal flu that usually complicates patients in the extreme age groups and who have significant comorbidity, the current pandemic has an unexpected tendency to complications in young and previously healthy people. The more severe cases are characterized by increased inflammatory markers, along with significant lymphopenia. As lymphopenia was associated with disease severity and its clinical course, we suggest using the lymphocyte count and its kinetics as a surrogate marker for disease severity and prognosis.

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