

Transient Lymphopenia and Neutropenia: Pediatric Influenza A/H1N1 Infection in a Primary Hospital in Israel

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ABSTRACT: **Background:** Pandemic influenza A/H1N1 carries a relatively high morbidity, particularly in young people. Early identification would enable prompt initiation of therapy, thereby improving outcomes.

Objective: To describe the epidemiological, clinical and laboratory characteristics of children admitted to hospital with the clinical diagnosis of influenza with reference to pandemic influenza A/H1N1.

Methods: We conducted a prospective study of all children aged 16 years or less admitted to the pediatric department with the clinical diagnosis of influenza-like illness from July to October 2009. The presence of A/H1N1 virus was confirmed using real-time reverse transcriptase polymerase chain reaction (RT-PCR) analysis of nasopharyngeal secretions. Positive cases were compared with negative cases concerning epidemiological data, risk factors, clinical presentation and laboratory parameters, with emphasis on changes in the differential blood count.

Results: Of the 106 study patients, 53 were positive to influenza A/H1N1 and 53 were negative. In both groups nearly all patients had fever at presentation and approximately two-thirds had both fever and cough. All patients had a mild clinical course, no patient needed to be admitted to the intensive care unit and no mortalities were recorded. Hyperactive airway disease was more common in the A/H1N1-positive group. Pneumonia occurred in 30% of children in both groups. Laboratory findings included early lymphopenia and later neutropenia in the A/H1N1-infected patients.

Conclusions: Leukopenia consisting of lymphopenia and later neutropenia was common in patients with A/H1N1 infection but was not correlated with disease severity or clinical course, which were similar in both groups. However, reduced leukocyte count can be used as an additional criterion for diagnosing A/H1N1 infection until RT-PCR results are available.

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KEY WORDS: influenza, leukocytes, lymphocytes, infection

In late March and early April 2009 an outbreak of a novel influenza virus designated A/H1N1 influenza (popularly known as swine flu) was reported in Mexico, with subsequent cases observed in many countries, leading to the declaration by the World Health Organization of a worldwide pandemic [1]. The report by Chowell et al. [2] emphasized two important features of the newly described pandemic strain, namely, a distinct shift in the timing of the disease and the age distribution of patients, with young adults becoming ill and dying mainly of respiratory illness. These two features were characteristics of previous pandemics [3].

The first case of influenza A/H1N1 in Israel was diagnosed at the end of April 2009 at Laniado Hospital in a young man returning from Mexico. By the end of November 2009, a total of 6870 cases of pandemic influenza A virus were laboratory-confirmed in Israel, with 50 deaths. Among the fatalities were three children: one premature infant with severe cardiopulmonary disease and two with neurological disease [4]. We examined the epidemiological, clinical and laboratory characteristics of patients who were admitted to our ward with the clinical diagnosis of an influenza-like illness, comparing those who tested positive for A/H1N1 virus with those who tested negative.

PATIENTS AND METHODS

The study was conducted between July and October 2009 in the pediatric ward of Laniado Hospital. All infants and children aged 0–16 years who were hospitalized with the clinical diagnosis of influenza-like illness were enrolled. Symptoms included fever $\geq 38^{\circ}\text{C}$ and cough, as well as sore throat, headache, myalgia, prostration and vomiting.

Influenza-like illness is defined by the U.S. Centers for Disease Control and Prevention as fever (temperature $\geq 100^{\circ}\text{F}$ or 37.8°C) and either cough or sore throat in the absence of another known cause. (CDC interim guidance on case definitions can be used for investigations on novel influenza A/H1N1). All patients with clinical features compatible with influenza infection who were tested for A/H1N1 virus were included in the study. We compared epidemiological, clinical

CDC = Centers for Disease Control

and laboratory data between those with positive and those with negative results. The following data were recorded for all patients: age, gender, duration of symptoms before admission, the presence or absence of preexisting illness, complications, and duration of hospital course.

LABORATORY ANALYSIS

Nasopharyngeal swabs were collected on admission, placed in transport medium and sent to the Central Virology Laboratory, Sheba Medical Center. All samples were tested for the presence of A/H1N1 virus using a real-time (RT-PCR) method. Results were obtained within 24 hours.

On the day of admission all patients had blood taken and analyzed for complete blood count. Patients with abnormal differential count had repeated samples taken at regular intervals. Additional tests included C-reactive protein, creatine phosphokinase, liver function tests and blood cultures. A chest radiograph was done and patients with evidence of pulmonary infiltration and/or consolidation were treated with antibiotics. Antibiotics were also given to those with other infections found on clinical examination, e.g., otitis media, tonsillitis, urinary tract infection. After nasopharyngeal samples were taken, selected patients were given oseltamivir according to Health Ministry guidelines and clinical presentation. The duration of treatment was determined by the RT-PCR results and continued for 5 days in the positive group; treatment was stopped in those who tested negative. The study was approved by the Ethics Committee of the hospital.

STATISTICAL ANALYSIS

A two-sample *t*-test was used to compare mean values and was adjusted for whether or not the standard deviations were similar. For non-parametric data the chi-square test was used. A *P* value < 0.05 was considered significant. We entered into a logistic regression model all variables that on univariate analysis had a *P* value < 0.1. The variables that did not add significantly to the model were removed one at a time, and only after those that added significantly to the model were included were the others added back one at a time, but retained only if they then added significantly to the model. The Hosmer-Lemeshow Statistic was calculated to determine if the model was acceptable, and the area under the curve was calculated to determine the differentiating ability of the model.

RESULTS

Of the 106 patients in the study 53 were positive for influenza A/H1N1 and 53 were negative. The median age of the patients was 8.2 ± 4.5 years in the A/H1N1-positive group and 3.5 ± 3.8 years in the A/H1N1-negative group. Preexisting conditions were present in 30 (56.6%) and 21 (39.6%) in the

positive and negative group respectively; 35.8% of patients in the positive group and 22.6% in the negative group had hyperactive airway disease (*P* = 0.06).

CLINICAL MANIFESTATIONS

Fifty-one patients (96%) in the positive group and 52 (98%) in the negative group had fever at presentation. Cough was present in 37 (69.8%) and 35 (66%) in the positive and negative group respectively. Other symptoms included rhinorrhea, vomiting and diarrhea. The clinical manifestations at presentation are listed in Table 1.

The majority of patients presented within 3 days of onset of symptoms: 73.5% and 60.3% in the positive and negative group respectively. There was no difference in time taken to seek medical attention between the two groups (*P* = 0.38).

LABORATORY AND RADIOGRAPHIC FINDINGS

Blood count analyses on admission revealed leukopenia in the A/H1N1-positive group compared to the A/H1N1-negative group (7604 ± 5344 cells/mm³ vs. 12,325 ± 6764 cells/mm³, *P* < 0.001). The leukopenia consisted of a significant lymphopenia with normal neutrophil count in the A/H1N1-positive group compared to the A/H1N1-negative group (1270 ± 808 cells/mm³ vs. 3217 ± 2613 cells/mm³, *P* < 0.001). Lymphopenia was evident 2–3 days from the onset of fever and lasted 2–3 days.

Table 1. Comparison of admission characteristics in those with and without A/H1N1 influenza infection

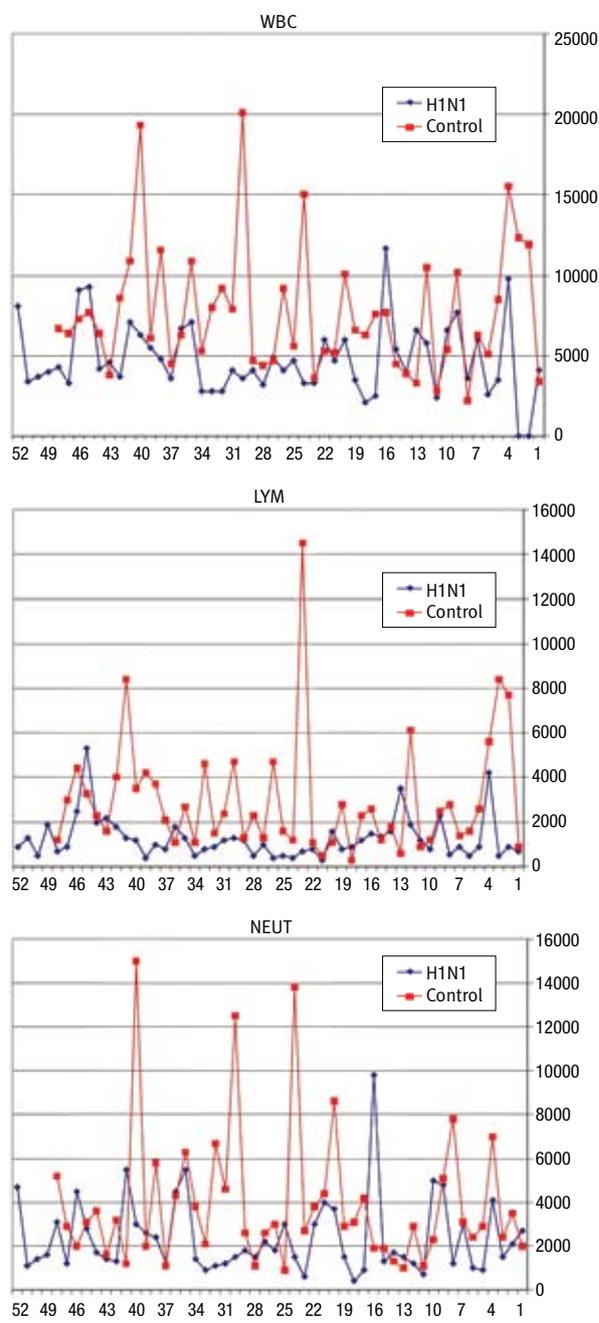
| | A/H1N1-positive N=53 | A/H1N1-negative N=53 | P value |
|---------------------------------|-------------------------|-------------------------|---------|
| Demographic data | | | |
| Age (yrs) | 8.2 ± 4.5 | 3.5 ± 3.8 | < 0.001 |
| 0–2 | 7 (13.2) | 28 (52.8) | |
| 2–5 | 6 (11.3) | 14 (26.4) | |
| 6–10 | 20 (37.7) | 8 (15.0) | |
| 11–16 | 20 (37.7) | 3 (5.6) | |
| Female gender | 29 (54.7) | 30 (56.6) | 0.85 |
| Preexisting diagnosis | 30 (56.6) | 21 (39.6) | 0.06 |
| Asthma | 19 (35.8) | 12 (22.6) | |
| Clinical symptoms | | | |
| Fever | 51 (96.2) | 52 (98.1) | |
| Cough | 37 (69.8) | 35 (66) | |
| Sore throat | 14 (26.4) | 2 (3.8) | |
| Rhinorrhea | 8 (15) | 8 (15) | |
| Vomiting | 14 (26.4) | 15 (28.3) | |
| Diarrhea | 1 (1.8) | 5 (9.4) | |
| Laboratory findings | | | |
| Leucocyte count/mm ³ | 7604 ± 5344 | 12,325 ± 6764 | < 0.001 |
| Neutrophils/mm ³ | 5845 ± 4655 | 8296 ± 6126 | 0.02 |
| Lymphocytes/mm ³ | 1270 ± 808 | 3217 ± 2613 | < 0.001 |
| Hemoglobin g/L | 12.4 ± 12.2 | 12.1 ± 1.1 | 0.31 |
| Platelet count k/μl | 221 ± 77 | 320 ± 155 | < 0.001 |
| Infiltration on chest X-ray | 21 (39.6%) | 21 (39.6%) | |
| Antibiotic treatment | 31 (58.5) | 33 (62.2) | |
| Oseltamivir treatment | 39 (73.6) | 13 (24.5) | |
| Sick days before admission | 3.0 ± 2.4 | 3.4 ± 2.6 | 0.38 |
| Days in hospital | 3.5 ± 1.5 | 3.3 ± 1.8 | 0.52 |

Values are presented as number and % of total

RT-PCR = reverse transcriptase-polymerase chain reaction

A decline in neutrophil count was observed in most patients on the fifth day after onset of fever when lymphocyte count showed a recovery. Normalization of neutrophil count was evident 2–4 days later, i.e., from day 7 to 10 after onset of symptoms [Figure 1].

Figure 1. Blood counts revealed leukopenia with initial lymphopenia and subsequent neutropenia in the majority of A/H1N1-positive patients, and counts within normal range in most patients in the A/H1N1-negative group.



Chest radiographs taken on admission showed pulmonary infiltrates in 39.6% of patients in both groups. Abnormalities ranged from scanty bilateral patchy infiltration to consolidation and pleural effusion. Antibiotics were administered to 31 patients in the positive group and to 33 in the negative group who developed pneumonia. Oseltamivir was given to 39 (73.6%) in the positive group and to 13 (24.5%) in the negative group.

CLINICAL COURSE AND OUTCOME

On average, patients in both groups presented on day 3 of illness (range 0.6–6). Duration of hospitalization was similar in both groups and averaged 3.5 days (range 1.5–5.1). All 106 patients recovered completely with no sequel related to A/H1N1 infection.

DISCUSSION

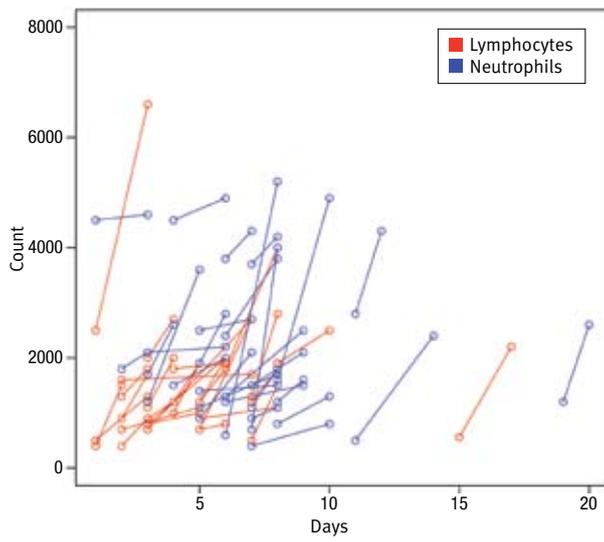
In our study we describe a cohort of 106 patients who presented with influenza-like symptoms and were tested for the presence of A/H1N1 virus. Using the RT-PCR method we found that 53 patients were positive and 53 negative. Unlike seasonal influenza that infects patients at the extremes of age, the pandemic A/H1N1 strain appears to attack infants, children and young adults, with people over 65 years old being relatively protected [5-8].

Our study population included 106 patients all under the age of 16. Seventy-five percent of patients in the positive group were between the ages of 6 and 16 years, whereas only 20.6% of patients in the negative group were in this age range. However, more than half of the patients in the negative group were under 2 years old. These findings are in agreement with other reports, namely that the age group at risk is young children and adolescents.

Hyperactive airway disease was the most important risk factor in developing A/H1N1 infection and was present in 19 (35.8%) and 12 (22.6%) of the positive and negative group respectively ($P = 0.06$). Similar findings were observed by O'Riordan et al. [9] who noted no relation to the severity of asthma and infection with A/H1N1.

The clinical manifestations of A/H1N1 virus infection range from self-limited uncomplicated subfebrile respiratory illness to severe and even fatal respiratory disease and extrapulmonary disease. Gastrointestinal symptoms include vomiting, diarrhea and abdominal pain. Neurological manifestations range from decreased mental status to seizures, encephalopathy or encephalitis [10,11]. Influenza virus infection has been associated with hematological abnormalities such as pancytopenia and leukopenia mainly due to lymphopenia. In our study the main clinical presentations [Table 1] were fever and cough. Less common manifestations included sore throat, rhinorrhea and vomiting.

Figure 2. Lymphopenia was evident 2–3 days from the onset of fever and lasted 2–3 days. A decline in neutrophil count was observed in most patients on the fifth day after onset of fever when lymphocyte count showed a recovery. Normalization of neutrophil count was evident 2–4 days later. i.e., from day 7 to 10 after onset of symptoms



HEMATOLOGICAL MANIFESTATIONS

Blood counts revealed leukopenia with initial lymphopenia and subsequent neutropenia in the majority of A/H1N1-positive patients and counts within normal range in most patients in the A/H1N1-negative group. The lymphopenia typically occurred on day 2 (range 1–3) and resolved by day 7 (range 6–9) [Figures 1 and 2]. These findings are similar to those described by Cao and co-authors [12], who found lymphopenia to be common in both adults (68.1%) and children (92.3%) infected with A/H1N1 virus. Lewis et al. [13] suggested that the lymphopenia is mainly due to reduction in T cells and to a lesser extent in B cells and is of short duration. Depletion of lymphocytes is observed with the onset of illness, and recovery is observed soon after the fever subsides. The lymphopenia typical of influenza A/H3N3 during acute illness was shown to be due to a reduction in both T and B cells without alteration in the CD4:CD8 ratio. This is in contrast to the findings of Cao et al. [12] who noted an abnormal CD4:CD8 ratio in half of those who were positive for A/H1N1.

Nichols and collaborators [14] proposed several mechanisms to explain influenza-induced lymphopenia: Lymphopenia could be the result of cell migration from the circulation and/or cell death caused by necrosis or by apoptosis through suppression of hematopoiesis. Apoptosis after exposure to influenza A virus could be a result of virus-induced cytokine stimulation or viral induction of Fas. In addition, increased natural killer cell activity in the periphery

Table 2. Linear regression model to predict H1N1 positive swabs

| | Odds ratio | 95% confidence interval | P value |
|-----------------------------------|------------|-------------------------|---------|
| Age (yrs) | 1.15 | 1.09–1.28 | < 0.001 |
| Lymphocytes (1000 cells/ μ l) | 0.49 | 0.36–0.68 | < 0.001 |
| Asthma | 3.34 | 1.06–10.50 | 0.039 |

Area under the curve 87.2%

may be reflective of increased NKC activity in the lung. The early lymphopenia and the later neutropenia in the influenza-infected patients may represent migration of these cells from the circulation to the infected respiratory tract as a consequence of infection.

Univariate analysis showed that those positive for A/H1N1 were older and had asthma more frequently. Laboratory findings in this group included leukopenia, lymphopenia and neutropenia, and thrombocytopenia [Table 1]. On multivariate analysis, the logistic regression model showed that older age, a history of asthma and a lower lymphocyte count predicted those with A/H1N1 [Table 2]. The model was acceptable with the Hosmer-Lemeshow statistic of 9.09 ($P > 0.334$) and had good differentiating ability with an area under the curve of 87.2%. These variables can be taken into account when differentiating A/H1N1 infection from other viral infections of the respiratory tract.

CONCLUSIONS

We found infection with A/H1N1 virus to be mild with an uneventful clinical course in most of our hospitalized patients. Asthma was a major risk factor. The striking laboratory finding was early lymphopenia and later neutropenia, which were transient and did not correlate with either the severity or the length of the clinical course.

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