Hematological consequences of pandemic influenza H1N1 infection: a single center experience

Şule Ünal[1], Müge Gökçe[1], Selin Aytaç-Elmas[1], Erdem Karabulut[2], İlhan Altan[1], Aslınur Özkaya-Parlakay[3], Ateş Kara[3], Mehmet Ceyhan[3], Ali Bülent Cengiz[3], Murat Tuncer[1], Mualla Çetin[1], Fatma Gümrük[1]

Units of [1]Pediatric Hematology, and [3]Pediatric Infectious Diseases, Department of Pediatrics, and [2]Department of Biostatistics, Hacettepe University Faculty of Medicine, Ankara, Turkey

SUMMARY: Ünal Ş, Gökçe M, Aytaç-Elmas S, Karabulut E, Altan İ, Özkaya-Parlakay A, Kara A, Ceyhan M, Cengiz AB, Tuncer M, Çetin M, Gümrük F. Hematological consequences of pandemic H1N1 infection: a single center experience. Turk J Pediatr 2010; 52: 570-575.

Since its identification in April 2009, pandemic influenza H1N1 virus has affected thousands of people worldwide. Viruses, particularly Epstein-Barr virus, cytomegalovirus and parvovirus B19, may have diverse hematological consequences, including anemia, neutropenia, thrombocytopenia, lymphocytosis, hemophagocytic lymphohistiocytosis, and coagulation abnormalities. In this study, a total of 31 consecutive pediatric patients, with and without chronic diseases, who had flu symptoms and were confirmed to have pandemic influenza, were evaluated for hematological consequences upon presentation to hospital. Eight (25.8%) patients had leukopenia and six (19.4%) had thrombocytopenia at the time of diagnosis of H1N1 infection. Pandemic influenza H1N1 infection may cause diverse hematological findings, including cytopenias and hemophagocytosis.

Key words: children, H1N1, pandemic influenza, hematological findings.

In April 2009, a novel H1N1 influenza A virus, the so-called pandemic influenza H1N1 virus, was identified in Mexico and has since spread throughout the world, causing an influenza pandemic as defined by the criteria of the World Health Organization (WHO). On 11 June 2009, the WHO issued a pandemic alert level of six (of 6 possible levels)[1]. As of 6 December 2009, more than 208 countries and overseas territories or communities worldwide have reported laboratory-confirmed cases of pandemic influenza H1N1 2009, including at least 9596 deaths[2].

The cases reported to date have generally been mild, recovering fully within one week, even in the absence of any medical treatment. The age distribution is typical for seasonal influenza, with school children representing the age group with the highest rates of infection[1,3]. However, pregnant women seem to be at an increased risk for complications from pandemic influenza H1N1 virus infection[4]. Other groups at an increased risk of severe or fatal illness include people with underlying medical conditions, most notably chronic lung disease (including asthma), cardiovascular disease, diabetes, and immunosuppression[5].

Various viruses, most notably Epstein-Barr virus, cytomegalovirus, parvovirus B19, human immunodeficiency virus, and Nairovirus, which is the etiologic agent of Crimean-Congo hemorrhagic fever, may cause a diverse group of hematological manifestations in humans, including anemia, neutropenia, thrombocytopenia, pancytopenia, leukocytosis, lymphocytosis, hemophagocytic lymphohistiocytosis, lymphoproliferative disorders, and coagulation abnormalities, such as disseminated intravascular coagulation[6-12].

Hematological consequences of viral infections usually appear in the early phase of the acute infection. There is limited data in the literature on the hematological effects of H1N1

infection. Herein, we report the hematological manifestations of a series of pediatric patients infected by pandemic influenza H1N1 virus.

Material and Methods

This study included a total of 31 consecutive pediatric patients who had flu symptoms between 13 July 2009 and December 2009 and were confirmed to have pandemic influenza H1N1 infection by two-step polymerase chain reaction from nasopharyngeal specimen method through nasopharyngeal aspirates. In the first step, a conservative section of influenza A and B viruses was used, and in positive specimens, a second step was performed with swine influenza A/California/04/2009 probe from the Centers for Disease Control and Prevention. A detailed history was obtained, including the presenting complaints, antipyretic use, and any underlying chronic disorders, including cardiac, hematological, pulmonary, neurological, and gastrointestinal systems. The hematological indices of patients at presentation to hospital were evaluated retrospectively. Of the 31 patients, 17 (54.8%) had an underlying chronic disorder (Table I). Fourteen (45.2%) patients had no underlying chronic disease and were otherwise healthy up to the H1/N1 infection (Group 1).

Of the 17 patients with an underlying chronic disorder, nine patients had a chronic disorder other than a disease that may affect bone marrow (Group 2) (von Willebrand disease (vWD) (n=2), unspecified mental motor retardation (n=2), cerebral palsy (n=1), spinal muscular atrophy (n=1), ileal atresia (n=1), asthma (n=1), and congenital heart disease (n=1)). Eight had a chronic disorder that may affect bone marrow (Group 3), including acute lymphoblastic leukemia (ALL) under chemotherapy (n=4), newly diagnosed acute myeloid leukemia (AML) with Bloom syndrome (n=1), newly diagnosed AML with Down syndrome (n=1), AML in which chemotherapy had been ceased 3 months before (n=1), and methylmalonic acidemia (n=1).

All of the patients had hemogram and peripheral blood smear. Four patients had bone marrow aspiration for intractable fever accompanying cytopenia(s). The peripheral smears and bone marrows were examined by the same hematologist. The four patients with bone marrow examination were additionally evaluated for hemophagocytic lymphohistiocytosis (HLH) with biochemical analyses including serum ferritin, lipid profile and plasma fibrinogen. Fisher's exact test was used to analyze the association between the

Table I. Clinical and Hematological Findings of the Patients Infected with Pandemic Influenza H1N1

	Group 1 (n=14)	Group 2* (n=9)	Group 3** (n=8)	Total (n=31)
Age	8.6±4.9 (7 mos-15 yrs)	5.8±5.4 (1-16 yrs)	8.7±5.6 (2-17 yrs)	7.8±5.2 years (7 mos-17 yrs)
Male/Female	8/6	5/4	7/1	20/11
Symptoms				
Fever	11/14 (78.6%)	8/9 (88.9%)	7/8 (87.5%)	26/31 (83.9%)
Cough	14/14 (100%)	9/9 (100%)	7/8 (87.5%)	30/31 (96.8%)
Sore throat	10/14 (71.4%)	2/9 (22.2%)	5/8 (62.5%)	17/31 (54.8%)
Hemoglobin (g/dl)	13.3±1 (11.5-15)	12.5±1.5 (10-14)	10.7±1.9 (7.9-13.3)	12.4±1.7 (7.9-15)
WBC ($x10^9$/L)	7.4±2.9 (3-12.5)	12.9±9.4 (2.7-32.9)	2.8±1.2 (1.5-4.4)	7.8±6.5 (1.5-32.9)
ANC ($x10^9$/L)	4.2-2.3 (0.7-8.1)	6.6±8.3 (0.4-28.1)	0.79±0.54 (0.32-1.6)	4±5.1 (0.32-28.1)
Thrombocyte count ($x10^9$/L)	248±103 (87-458)	297±100 (161-493)	155±120 (7-320)	238±117 (7-493)

WBC: White blood cell count. ANC: Absolute neutrophil count.

*von Willebrand disease (vWD) (n=2), unspecified mental motor retardation (n=2), cerebral palsy (n=1), spinal muscular atrophy (n=1), ileal atresia (n=1), asthma (n=1), congenital heart disease (n=1)

**ALL under chemotherapy (n=4), newly diagnosed acute myeloid leukemia (AML) with Bloom syndrome (n=1), newly diagnosed AML with Down syndrome (n=1), AML whose chemotherapy had been ceased 3 months previously (n=1), methylmalonic acidemia (n=1)

presence of an underlying chronic disorder and hospital admission with the development of cytopenias.

Results

The mean age of the study group was 7.8±5.2 years (7 months-17 years) and 20 (64.5%) of the patients were males. The presenting complaint was cough in 30 (96.8%), fever in 26 (83.9%) and sore throat in 17 (54.8%) (Table I).

Of the 26 patients who had fever, 14 (53.8%) had fever more than three days at presentation and 14 (53.8%) had fever above 38.5°C. Of the 26 patients who had fever, 21 (80.8%) received paracetamol and 5 (19.2%) received ibuprofen prior to presentation. All of the 8 leukopenic patients had received antipyretic prior to presentation at hospital, and 7 and 1 of these patients had used paracetamol and ibuprofen, respectively. Nine of 10 patients who developed neutropenia had a prior history of antipyretic use (8 paracetamol, 1 ibuprofen). It was learned that all of the patients who developed thrombocytopenia had used paracetamol prior to hemogram analyses.

The hematological findings of the 31 patients are summarized in Table I. Eight (25.8%) patients had leukopenia and 6 (19.4%) had thrombocytopenia at the time of diagnosis of H1N1 infection (Table I). Leukopenic patients had a mean white blood cell count (WBC) of 2.3±0.6 $x10^9$/L (1.5-3.2 $x10^9$/L). Mean platelet count was 76.1±47.7$x10^9$/L (7-135$x10^9$/L) in thrombocytopenic patients. Absolute neutrophil count (ANC) was below 1.5$x10^9$/L in 10 (35.2%) of the patients. ANC was below 1$x10^9$/L and 0.5 $x10^9$/L in 8 (25.8%) and 5 (16.1%) of the patients, respectively.

Of the 21 patients who received paracetamol, 7 (33.3%) had leukopenia and 6 (28.6%) had thrombocytopenia, whereas of the 5 patients who received ibuprofen, 1 (20%) had leukopenia and none had thrombocytopenia.

In Group 1, the mean values of WBC, ANC and thrombocytes in leukopenic, neutropenic and thrombocytopenic subgroups were 3.1±0.14$x10^9$/L (3-3.2), 0.93±0.24 $x10^9$/L (0.76-1.1) and 87 $x10^9$/L (1 patient), respectively. In Group 2, the mean values of WBC and ANC in leukopenic and neutropenic subgroups were 2.7$x10^9$/L (1 patient) and 0.8±0.56 $x10^9$/L (0.4-1.2), respectively. In Group 3, the mean values of WBC, ANC and thrombocytes in leukopenic, neutropenic and thrombocytopenic subgroups were 1.9±0.5$x10^9$/L (1.5-2.7), 0.5±2 $x10^9$/L (0.3-0.9) and 74±53 $x10^9$/L (7-135), respectively (Table I). The presence of an underlying chronic disorder affecting bone marrow was not found to be statistically significantly associated with the development of cytopenias ($p>0.05$).

Of the 31 patients, 8 (25.8%) were lymphopenic and 5 (16.1%) exhibited monocytosis. None of the patients had a thrombocyte count higher than 500 $x10^9$/L. None of the patients had anemia related to hemolysis, and anemia could be attributed to iron deficiency or anemia of chronic disease in all patients; no direct relation between anemia and H1N1/09 could be established. Reticulocyte count was normal in all patients of the study group. Ten (35.2%) of the patients had pneumonia at the time of presentation. All of the patients received oseltamivir, and in 2 cases, it had already been initiated in local hospitals prior to admission. Of these 2 patients, 1 is the patient with AML whose chemotherapy had been ceased three months prior to H1N1 infection, and the other patient had ALL and was under chemotherapy. Both of these patients who received oseltamivir prior to presentation in our center had leukopenia, neutropenia and thrombocytopenia. Five (50%) of the 10 neutropenic patients received granulocyte colony-stimulating factor for a rapid recovery of neutropenia during H1N1 infection. Sixteen (51.6%) patients were hospitalized, and 1 required positive pressure ventilation. There was a statistically significant relation between hospital admission and presence of thrombocytopenia at presentation ($p=0.018$); however, leukopenia did not contribute to hospital admission ($p=0.22$).

Four patients with persistent fever and cytopenia(s) had bone marrow aspiration examination. Of these, 1 had a new diagnosis of AML (the patient with Down syndrome), and 1 ALL and 1 AML patient had no hemophagocytosis and were in remission. One 14-year-old patient who had no underlying chronic disorder and required subsequent ventilatory support was found to have extensive

hemophagocytosis, and with the help of biochemical analyses, she was determined to have 4/8 of the HLH criteria; however, studies for natural killer (NK) activity and soluble interleukin (IL)-2 receptor levels were unavailable, and HLH-2004 protocol13) was initiated. Fever subsided with the initiation of steroid, and the pulmonary fields, which were compatible with acute respiratory distress syndrome on chest X-rays, recovered by the second dose of etoposide. The patient is currently being weaned from the assisted ventilation temporarily.

Discussion

Viruses may cause cytopenias through various mechanisms, including decreased production, which may be due to concomitant use of drugs, viral suppression of bone marrow, inhibitory effects of the inflammatory cytokines, bone marrow necrosis or increased loss including hemolysis, immune thrombocytopenic purpura, thrombotic thrombocytopenic purpura, or hemophagocytosis[9]. The cytopenias in pandemic influenza H1N1 virus may be related to extensive use of antipyretics, especially non-steroidal anti-inflammatory drugs; however, in our study group, only one of the patients who developed cytopenia had a history of ibuprofen use. Additionally, cytopenia may be related to bone marrow suppression of the virus or hemophagocytosis. In six of the patients, cytopenia may have been related to the newly diagnosed AML (n=2) or preceding chemotherapy related to underlying ALL (n=4), which precludes attributing the cytopenias in these patients solely to the influenza virus.

However, the patients with chronic disease not affecting bone marrow (Group 2) and patients without any chronic disease (Group 1) also exhibited leukopenia, neutropenia and thrombocytopenia, indicating the hematological influences of pandemic influenza H1N1 virus (Table II).

The high rate of underlying chronic disease in the study group may be related to the higher sensitivity of families of patients with chronic disease to flu symptoms. On the other hand, patients with chronic disorders, especially chronic lung disease (including asthma), cardiovascular disease, diabetes, and immunosuppression are more prone to severe illness[5], which may have caused the more severe cases to admit to our tertiary center and the less severe cases, without any underlying disorder, to be managed at home or in primary care facilities.

To our knowledge, the only reported data about the cytopenias related to H1N1 in the literature comes from 11 patients (median age: 10 years, range: 16 months to 48 years), and of these 11 patients, complete blood counts were available for four patients, revealing leukopenia in two, lymphopenia in one, and thrombocytopenia in one[14].

In one of our cases, the development of HLH responsive to HLH-2004 protocol[13] including steroid, etaposide-16 and cyclosporine A was remarkable. Recurrent virally associated HLH was previously reported in a 15-month-old girl with disseminated Langerhans cell histiocytosis during maintenance therapy. Viral infection with influenza A, herpes simplex, and adenovirus, respectively, was reported to be documented at each episode[15]. In 2006, Henter et al.[16] hypothesized that avian influenza A virus subtype H5N1 infection, which had a very high mortality of 50%, could be treated with HLH therapy, taking into account the post-mortem analyses findings in affected patients, who were revealed to have hemophagocytosis similar to that found in patients with HLH. The relative improvement in pulmonary findings and recession of fever in our patient may be an encouraging example of the new approach suggested by Henter et al.[16].

In conclusion, pandemic influenza H1N1 infection may cause diverse hematological

Table II. Hematological Abnormalities of Patients According to Underlying Disorder

	Group 1 (n=14)	Group 2 (n=9)	Group 3 (n=8)
Leukopenia n (%)	2 (14.3%)	1 (11.1%)	5 (62.5%)
Neutropenia n (%)	2 (14.3%)	2 (22.2%)	6 (75%)
Thrombocytopenia n (%)	1 (7.1%)	-	5 (62.5%)

findings, including cytopenias and hemophagocytosis in patients both with and without an underlying disorder. Knowledge about the hematological effects of pandemic influenza H1N1 virus may increase the physicians' suspicion for the virus in a patient with flu-like symptoms. The patients with underlying leukemia seem to be not only prone to H1N1 infection related to their immunocompromised status, but also to the hematological manifestations, which may not be attributed solely to H1N1 virus. However, pandemic influenza H1N1 virus may have a more suppressive impact on the marrow of these patients related to the already injured status of their hematological progenitors. Hemophagocytosis should be considered in the course of infection with H1N1 infection; HLH developed in this series in a previously otherwise-healthy child. Early diagnosis of HLH and initiation of specific HLH treatment may improve the survival in potentially fatal severe cases.

Addendum:

We experienced two fatal cases of pandemic 2009 H1N1 infection in our center subsequent to the manuscript submission. One of these patients was a newly relapsed five-year-old boy with ALL who developed pneumonia and required ventilatory support related to acute respiratory distress syndrome. The other, a five-year-old male patient, was on maintenance treatment for ALL and developed HLH during the severe clinical course of pandemic 2009 H1N1 infection. He developed multi-organ failure, including respiratory and renal insufficiency, and was placed on ventilatory support, in addition to antibacterial and oseltamivir treatments. The patient's clinical condition worsened and HLH-2004 protocol was initiated; however, this treatment was also unsuccessful. Both patients were febrile with neutropenia and thrombocytopenia upon admission for H1N1 infection.

Pandemic 2009 H1N1 virus may have a more suppressive impact on the marrow of these patients related to the already injured status of their hematological progenitors. Hemophagocytosis should be considered in the clinical course of these patients. Our experiences indicate that the clinical course of H1N1 infection may be mild to severe, including HLH and fatalities on the latter end of the spectrum. The variable clinical course may be related to additional host factors including the status of the underlying disorder.

REFERENCES

1. Michaelis M, Doerr HW, Cinatl J Jr. An influenza A H1N1 virus revival - pandemic H1N1/09 virus. Infection 2009; 37: 381-389.
2. World Health Organization. Pandemic (H1N1) 2009-update 78. www.who.int/feeds /entity /csr/disease/swineflu/en/rss.xml (accessed Dec 12, 2009).
3. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med 2009; 360: 2605–2615.
4. Jamieson DJ, Honein MA, Rasmussen SA, et al.; Novel Influenza A (H1N1) Pregnancy Working Group. H1N1 2009 influenza virus infection during pregnancy in the USA. Lancet 2009; 374: 451–458.
5. World Health Organization. www.who.int/csr/ disease/ swineflu/ notes/h1n1_pregnancy_ 20090731/en/ index. html).
6. Walter RB, Hong TC, Bachli EB. Life-threatening thrombocytopenia associated with acute Epstein-Barr virus infection in an older adult. Ann Hematol 2002; 81: 672-675.
7. Giller RH, Grose C. Epstein-Barr virus: the hematologic and oncologic consequences of virus-host interaction. Crit Rev Oncol Hematol 1989; 9: 149-195.
8. Aslam M, Anderson JL, Guglietti D, Cardwell D. CMV-induced neonatal thrombocytopenia: a case report and review of the literature. Am J Perinatol 2007; 24: 429-434.
9. Hematological manifestations of systemic diseases. Infections. In: Nathan DG, Oski FA (eds). Hematology of Infancy and Childhood (7th ed). Philadelphia: Saunders Elsevier; 2009: 1696-1705.
10. Mustafa MM, McClain KL. Diverse hematologic effects of parvovirus B19 infection. Pediatr Clin North Am 1996; 43: 809-821.
11. Volberding PA, Baker KR, Levine AM. Human immunodeficiency virus hematology. Hematology Am Soc Hematol Educ Program 2003; 294-313.
12. Duru F, Fisgin T. Hematological aspects of Crimean-Congo hemorrhagic fever. Turk J Hematol 2009; 26: 161-166.
13. Henter JI, Horne A, Aricó M, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007; 48: 124-131.
14. Shinde V, Bridges CB, Uyeki TM, et al. Triple-reassortant swine influenza A (H1) in humans in the United States, 2005-2009. N Engl J Med 2009; 360: 2616.

15. Klein A, Corazza F, Demulder A, Van Beers D, Ferster A. Recurrent viral associated hemophagocytic syndrome in a child with Langerhans cell histiocytosis. J Pediatr Hematol Oncol 1999; 21: 554-556.

16. Henter JI, Chow CB, Leung CW, Lau YL. Cytotoxic therapy for severe avian influenza A (H5N1) infection. Lancet 2006; 367: 870-873.