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AN ISOLATED OUTBREAK OF INFLUENZA A H1N1 IN A HAEMATOLOGICAL DEPARTMENT DURING POST-PANDEMIC PERIOD

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ABSTRACT

INTRODUCTION AND OBJECTIVE. Influenza A H1N1 virus strain was associated with the pandemic outbreak of febrile respiratory infections worldwide in 2009, however in August 2010, the WHO announced that the world had entered the postpandemic period. It offered specific recommendations for this period, including the identification of clusters of severe respiratory disorders and deaths. Here we report the fulminant course of influenza A H1N1 infection in the postpandemic period in a group of patients in a single hematology department. We make an attempt to identify potential risk factors and the mode of spreading, and to provide recommendations for best practice.
MATERIAL AND METHODS. We conducted a retrospective analysis of a cluster of patients diagnosed with or suspected of influenza A H1N1 infection in the period from December 2010 to March 2011.
RESULTS. Fourteen patients with hematological disorders unexpectedly developed acute respiratory failure ARDS (Acute Respiratory Distress Syndrome). Of them, nine tested positive for influenza A H1N1 in a screening test and eight in confirmatory polymerase chain reaction. The infection was fatal in nine patients, despite artificial ventilation in eight and oseltamivir administration in 11. Ten were in reverse isolation according to CDC. No similar cases occurred in the whole hospital concurrently, or in the hematology wards at any other time.
CONCLUSIONS .The occurrence of A H1N1 epidemics in a hematological ward in the post-pandemic period highlights the importance of awareness of this complication, prompt testing and antiviral treatment. Furthermore, it confirms the importance of vaccinating patients and personnel against influenza as a prophylactic measure.

Key words: *influenza A H1N1, disease outbreak, complications*

INTRODUCTION

In 2009, the influenza A H1N1 virus strain was associated with the outbreak of febrile respiratory infections worldwide, as declared by the World Health Organization (WHO). H1N1 infections were reported in more than 214 countries; there were more than 500,000 cases and 18,849 deaths (as of 1 August 2010) (1). In Poland, 400 laboratory-confirmed cases were reported from May 2009 to September 2010, resulting in 131 deaths (2). In August 2010, the WHO announced that the world had entered the postpandemic period and offered specific recommendations for action that should be taken during this period, including the identification of clusters of severe respiratory disorders and deaths (3). These recommendations were adopted in Poland. According to the data of the National Institute of Public Health, Warsaw, Poland, from the 6th of September 2010 to the 27th of March 2011, 226 A/H1(v) infections were confirmed in Poland. The data did not reveal either an increased frequency or severity of respiratory tract infections in the general population in this period (2).

However, unexpectedly, from December 2010 to March 2011, a cluster of influenza A H1N1-positive cases occurred in a single hematology department. Herein, we describe and analyse this cluster.

MATERIALS AND METHODS

The study was approved by Medical University of Warsaw Review Board.

Patients. Influenza A H1N1 infection with a fulminant course was diagnosed or strongly suspected in 14 hematological patients in the Department of Hematology, Oncology and Internal Diseases, Medical University of Warsaw (Warsaw, Poland) between December 2010 and March 2011. During this period, 358 patients were hospitalised in our unit (190 female, 168 male; median age: 62 years, range: 18–97 years). Concurrently, only four patients in all other hospital departments (992 beds, 12,748 hospitalisations) tested positive for H1N1 by polymerase chain reaction (PCR) and experienced only mild symptoms. There were no cases with a similar clinical course that were not tested for H1N1. Furthermore, there was no influenza A H1N1 epidemic in Warsaw or the rest of Poland at this time.

The Division of Hematology. The Division of Hematology, in which the influenza outbreak occurred, comprises three wards adjacent to each other but with limited traffic between them. Ward A has 17 beds, Ward B 16 and Ward C 26. Each has separate nursing staff and two (Wards A and B) follow the procedures of reverse isolation. However, patients were admitted to these wards without quarantine providing they were asymptomatic for acute infections. In Ward C, there was open access for families and patients could walk freely outside the ward, including to the hospital cafeteria.

Patient observation. Patients with symptoms that suggested a respiratory tract infection, including fever, dyspnoea, cough, and radiological signs suggesting interstitial pneumonia in high resolution computed tomography underwent a screening test for influenza A after the first patient tested positive for influenza A H1N1 (the second patient in the described cohort). Confirmatory PCR for influenza A H1N1 was performed in all patients who tested positive in the screening test. PCR was also performed for some patients whose screening test was negative but whose symptoms strongly suggested influenza infection. Other necessary blood tests and scans were performed, depending on particular circumstances and indications. PCR testing for the clearance of viruses from respiratory secretions after the symptoms of infection had been eliminated was not performed routinely. Tests used for laboratory diagnosis of influenza A H1N1 virus infection

Influenza screening test. The Influenza A/B 2 Panel Test 4A470 by Gecko Pharma Vertrieb GmbH (Ahrensburg, Germany) was used for the initial diagnosis of influenza. The sensitivity of the test is 76.3%, while the specificity 92% according to the manufacturer.

Polymerase chain reaction test. Mucosal smears from nose and throat were the source of viral genetic material. Nucleic acids were extracted from the samples using a High Pure Viral Nucleic Acid Kit (Roche Applied Science, Penzburg, Upper Bavaria, Germany) according to the manufacturer's instructions. Real-time reverse transcription (RT)-PCR was performed using a Quantification of Swine H1N1 Influenza Human Pandemic Strain Advanced Kit and Precision OneStep™ qRT-PCR MasterMix (PrimerDesign Ltd., Southampton UK). The reaction mix was prepared according to the manufacturer's instructions with minor modifications. A *β-actin* gene fragment was amplified for each sample to confirm the extraction of a valid biological template and to exclude PCR inhibition. One-step real-time RT PCR was performed in a Light Cycler® 2.0 (Roche Applied Science). Reverse transcription (55°C, 10 minutes) and enzyme activation (95°C, 8 minutes) were followed by 50 cycles of denaturation at 95°C for 10 seconds and primer annealing and extension at 60°C for 1 minute; data was collected at the end of the final step.

Method of retrospective analysis. The medical records of patients diagnosed with or suspected of having influenza A H1N1 were analysed to collect data on the type and status of underlying primary hematological disorder, complete blood count abnormalities, complications, effect of treatment on survival, and overall survival. In addition, the means of transmission was investigated.

Statistical analysis. STATISTICA 9.0 software (StatSoft, Inc., Tulsa, OK, USA) was used for basic calculations. Continuous variables are expressed as the median and range. No statistical tests were performed, because of the small sample size.

RESULTS

Of the 14 patients analysed, eight tested positive for influenza A H1N1 by PCR and six either were not tested in the initial phase of the outbreak, or tested negative despite presenting symptoms that strongly suggested influenza. PCR-confirmed patients are described separately from other patients. None of the patients had been vaccinated against influenza in the preceding year. Of the hospital personnel, about half of the nurses and the majority of doctors were vaccinated, but unvaccinated personnel worked on every ward.

The timing and mode of influenza A H1N1 transmission. Probably the first cases of influenza occurred throughout January and at the beginning of February 2011. Initially, two patients (one male, one female) with no formal confirmation of influenza developed respiratory insufficiency. In one, no diagnostic tests were performed; in the other, negative screening results were obtained and PCR testing was not performed. They were hospitalised in the reverse isolation ward (A) in our division, as described in the Patients and Methods.

Both patients required mechanical ventilation for respiratory failure. The clinical course in the male patient (with negative screening results) was complicated by intracranial hemorrhage and multiorgan failure with disseminated intravascular coagulation in the early post transplantation period. In the female patient, respiratory insufficiency remained the primary problem.

At the end of January, two new cases occurred in the second (B) reverse isolation ward (one confirmed, one highly suggestive; both negative on screening tests; both with severe respiratory insufficiency that required mechanical ventilation following clinically overt extensive bleeding in the respiratory tract. Approximately 1 week later, a new case was diagnosed in the free-access ward (C). Notably, at the same time, another patient visited an outpatient unit located on the same floor and died shortly afterwards from respiratory failure, in a regional hospital.

The two patients in whom influenza was suspected had been hospitalised in a reverse isolation unit for a long time, so it is unlikely that they were infected by other patients. There were following possibilities of occurrence of such infections, either (i) patients were infected by asymptomatic staff, or (ii) they had latent infections and the pathogens were activated as a result of decreased immunity, or (iii) they were infected in the out-hospital environment and admitted to the hospital before development of symptoms, which could be the case of a female patient. It is worth mentioning, that during that period there was no increase in influenza incidence in the general population.

The second outbreak of influenza occurred in early March 2011, with the majority of cases being diagnosed in Ward B (four PCR-confirmed cases in two adjacent rooms), one in reverse isolation Ward A and two (positive screening, negative PCR) in free-access Ward C.

Representative case. A 48-year-old woman was admitted to our department in December 2010 with relapsed acute myeloid leukaemia (AML) following allogeneic hematopoietic stem cell transplantation (SCT). Flow cytometry of the cerebrospinal fluid revealed the presence of blasts, which indicated treatment with high-dose cytosine arabinoside. During the pancytopaenia period following treatment, the patient experienced fever but there was no obvious local infection. She received broad-spectrum antibiotics and was tested for influenza, with a negative result. High-resolution computed tomography was performed to locate the cause of fever; this revealed ground-glass opacities, which suggested alveolar hemorrhage. Initially, the patient did not complain of dyspnoea, but her condition deteriorated within hours and blood gas analysis revealed respiratory failure, with an arterial oxygen partial pressure of 36.5 mm Hg. Oxygen therapy and glucocorticosteroids were initiated. Mechanical ventilation was implemented 4 days later for respiratory arrest. Despite intensive antibiotic and symptomatic treatment, the patient's condition did not improve: she bled actively from the respiratory tract and blood gas analysis showed increasing CO_2 retention, with continuously low O_2 pressure. Diagnostic tests for influenza were repeated and both screening and confirmatory PCR tests were positive. The patient received oseltamivir at a standard dose administered through nasogastric feeding tube from the day of positive screening results, which was 7 days after initiation of mechanical ventilation. However, her status deteriorated and she died on the next day, with signs of bleeding in the respiratory tract. It is worth mentioning that the patient was the first in the analyzed cohort with confirmed A H1N1 infection.

Patients with confirmed influenza A H1N1 infection. Influenza A H1N1 infection was confirmed in two males and six females (median age: 57.5 years). Four patients had AML (one in first complete remission during consolidation chemotherapy, two during induction chemotherapy and one in relapse after allogeneic SCT); two had acute lymphoblastic leukaemia (ALL; 77 and 155 days, respectively, after allogeneic SCT); one had myelodysplastic syndrome with accompanying tuberculosis; and one had light chain amyloidosis with heart involvement, treated by autologous peripheral blood SCT.

The median time from the first onset of symptoms to the diagnosis of influenza was 3 days (range: 0–11 days). One patient was asymptomatic. The time from the onset of symptoms to the start of treatment with oseltamivir ranged from 0 to 11 days (median: 2 days).

At diagnosis, three patients had neutropaenia <0.5 G/l, three had a neutrophil count from 0.5–1.5 G/l and two had a normal neutrophil count. Five patients had lymphopaenia <0.8 G/l (Grade ≥2 based on the Common Terminology Criteria for Aderse Events version 3.0 (4) prior to the onset of influenza, while only three had lymphopaenia below 0.3 G/l (as defined in the work of *Ljungman* (5)). The median duration of lymphopaenia <0.8 G/l was 10 days (range: 3–25 days) and 0 days (range: 0-25 days) for lymphopaenia <0.3 G/l. Seven patients were thrombocytopaenic (median: 27 G/l; range: 2–68 G/l) and seven were anaemic (median: 9.7 g/dl; range: 6.8–10.6 g/dl). All patients except one had elevated acute-phase proteins (C-reactive protein). Four patients had concomitant respiratory disease (pneumonia of different aetiology). High-resolution computed tomography revealed ground-glass opacities, alveolar opacities, thickening of the septa and possible alveolar hemorrhage. Six patients developed respiratory insufficiency; four of these required mechanical ventilation. The major problem in these patients was CO_2 retention. In addition, three patients experienced renal failure (one requiring hemodialysis) and four experienced hypotension and septic shock.

Five patients died as a result of complications to their underlying hematological disease that were due to influenza. The cause of death was multiorgan failure in two patients, septic shock caused by *Enterococcus* species in one, respiratory system hemorrhage in one and sudden cardiac death in one patient who was suffering from amyloidosis with heart involvement. Three patients who survived received oseltamivir for 11–17 days (median: 11 days). A clearance PCR test was performed in only one patient and was negative after treatment was stopped.

Notably, four nonhematological patients who were confirmed by PCR to have influenza A H1N1, treated outside our division in the same hospital at approximately the same time, and treated in internal medicine departments rather than in intensive care units recovered quickly. All of these were discharged after 5–14 days of hospitalisation.

Patients with symptoms that suggested influenza infection, i.e. unexplained fever, dyspnoea, cough, bleeding in the respiratory tract and respiratory insufficiency. Four male and two female patients had such symptoms. Their median age was 49 years (range: 41–69) and were suffering from the following diseases: AML, ALL, multiple myeloma, chronic myelomonocytic leukaemia, T-cell prolymphocytic leukaemia and diffuse large B-cell lymphoma. Two patients showed symptoms that suggested influenza after allogeneic SCT (during the procedure and 1 year thereafter) and one following autologous peripheral blood SCT (+14 days). Two patients had a positive screening test but negative confirmatory PCR; one had a negative screening test with no PCR; one did not have any tests performed for technical reasons; and one died of fulminant respiratory failure shortly after visiting an outpatient unit in a regional hospital.

Before the onset of symptoms that suggested influenza, five patients experienced lymphopaenia <0.8 G/l and two <0.3 G/l. The median duration of lymphopaenia <0.8 G/l was 3 days (range: 2–19 days), while for <0.3 G/l it was 0 days (range: 0-12 days). At the onset of infection, two patients were severely neutropaenic (0 G/l), one had a neutrophil count of 0.7 G/l and two had a normal neutrophil count. Five patients were thrombocytopaenic (median: 34 G/l; range: 4–53 G/l) and five were anaemic (median hemoglobin concentration: 8.7 g/dl; range: 8.6–11.3 g/dl).

Five patients required oxygen therapy for respiratory insufficiency; four of these required mechanical ventilation. Similarly to the 'confirmed group', one patient developed renal failure and one patient bled from the respiratory system.

Four patients died in the course of suspected influenza: three from respiratory failure and one from multiorgan failure. Both surviving patients had a positive screening test (one was lost to follow-up after several months).

DISCUSSION

We have described the dramatic course of influenza A H1N1 infection in the postpandemic period in a group of patients in a single hematology department. It is important to stress that, at the time, there was no outbreak of influenza in the general population. Furthermore, when there was an outbreak of influenza A H1N1 in the season 2009-2010 in the general population, we experienced only a few cases with a benign course and a similar situation did not occur during the subsequent 18 months.

It is not possible to establish the origin of infection in particular patients, but three mutually compatible possibilities must be considered. Firstly, patients could have been infected asymptomatically prior to admission and have developed full-blown disease after chemotherapy or as the result of immunodeficiency in the course of their underlying blood disorder. Secondly, they could have been infected by infected personnel who were asymptomatic, particularly those who violated isolation procedures. Thirdly, patients could have been infected by each other, especially as there was no contact isolation. None of these possibilities can be excluded and more than one may well have been operative. It is highly unlikely that there was a single point of origin for the outbreak, for example, a single infected patient or member of staff who was case zero, because patients and personnel in different wards do not have contact with each other. Only during the second outbreak of influenza the majority of affected were patients hospitalised in the reverse isolation unit (B); patients were housed in adjacent rooms and allowed to leave them, which rendered it possible for them to infect each other. That patients infected one another is very likely in this situation.

Although the virus usually causes acute infection and does not persist in infected individuals (6), the activation of latent infection cannot be excluded in at least some immunocompromised patients. There is little published data on A H1N1 virus biology in severely immunocompromised patients, e.g. after hematopoietic stem cell transplantation, but on the basis of such data as there is, some authors suggest prolonged viral shedding (7,8). *Souza* et al. (9) showed that the median duration of viral shedding in a population of influenza A H1N1-infected cancer patients receiving immunosuppression was 23 days, with the full range being from 11 to as many as 63 days. Therefore, in our opinion, it is possible that some patients acted as reservoirs for the virus. There is no way of determining the relative likelihood that the virus was spread by asymptomatic patients or personnel, or by patients who were already showing symptoms (the third possibility). However,

in the absence of such determination, these possibilities must be afforded equal consideration. Under such circumstances, it seems worth considering whether it would be economically feasible to test all patients and medical staff entering these wards each day, especially during the typical influenza season.

Defining risk factors for severe or fatal influenza infection in hematological patients is crucial. Unlike *Ljungman* et al. (5), who found lymphopaenia to be an important risk factor for death in stem cell transplant recipients with influenza A H1N1 infection, lymphopaenia, defined both as below 0.8 G/l and below 0.3 G/l, did not seem to affect survival in our patients. However, there were only two SCT recipients in our group, the majority of whom were nontransplanted hematological patients, so the groups of patients were not strictly comparable. Thrombocytopaenia was observed to affect survival time adversely in our patients, a phenomenon also observed by *Cordero* et al. (10). In our patients, none with a platelet count of <50 G/l survived the acute phase of infection. This may be explained by fairly frequent alveolar bleeding that complicated a lower respiratory tract infection, which led to respiratory insufficiency and finally death. Severely anaemic patients also died more frequently in our group. Notably, we observed hypoxemia in 66.7%, in contrast to *Cordero* et al. (10), who observed it in only 10.7% of immunosuppressed infected patients. In summary, the principal risk factors seem to be thrombocytopaenia and anaemia.

It is extremely difficult to diagnose acute influenza infection in the postepidemic/ postpandemic period in immunocompromised patients, who frequently develop neutropaenic fever and are able to develop infection with almost any known pathogen. Nevertheless, hematologists should be encouraged to stay alert to the possibility of influenza infection, especially in febrile patients with respiratory tract infections, and to initiate antiviral treatment without delay. *Cordero* et al. (10) showed that prompt antiviral treatment improves survival rates. Even when the vaccination rate in the general population is low (11) vaccination should be recommended to health-care workers, families of patients and immunocompromised patients, because of the potential health gains, however limited these may be (12-14).

SUMMARY AND CONCLUSIONS

We described an unusual outbreak of influenza A H1N1 in a single hematology department, which could not be predicted by the situation in the country general population and is not a standard infectious risk in the care of hematological patients. Nine of the 14 affected patients died.

Although there is, as yet, no empirical confirmation, the risk of such an outbreak could be reduced by routine vaccination of hematological patients, their families and personnel against influenza. Moreover, awareness among doctors, immediate testing and prompt administration of oseltamivir could reduce the frequency of fatal complications.

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REFERENCES

1. World Health Organization. Pandemic (H1N1) 2009 - update 112. 2010 Aug [cited 2013 March 5]. Available from: http://www.who.int/csr/don/2010_08_06/en/index.html.
2. National Institute of Public Health. Influenza and influenza-like illness in Poland. 2013 [cited 2013 January 5]. Available from: http://www.pzh.gov.pl/oldpage/epimeld/grypa/aindex.htm.
3. World Health Organization. WHO recommendations for the post-pandemic period. 2010 Aug [cited 2013 January 5]. Available from: http://www.who.int/csr/disease/swineflu/notes/briefing_20100810/en/index.html
4. Blood/Bone Marrow. Common Terminology Criteria for Adverse Events v3.0 (CTCAE), 2006: 4.
5. Ljungman P, de la Camara R, Perez-Bercoff L, et al. Outcome of pandemic H1N1 infections in hematopoietic stem cell transplant recipients. Haematologica 2011; 96: 1231-1235.
6. Hayden FG, Belshe R, Villanueva C, et al. Management of influenza in households: a prospective, randomized comparison of oseltamivir treatment with or without postexposure prophylaxis. J Infect Dis 2004; 189: 440-449.
7. Centers for Disease Control and Prevention (CDC) Oseltamivir-resistant novel influenza A (H1N1) virus infection in two immunosuppressed patients - Seattle, Washington, 2009. MMWR Morb Mortal Wkly Rep 2009; 58: 893-896.
8. Weinstock DM, Gubareva LV, Zuccotti G. Prolonged shedding of multidrug-resistant influenza A virus in an immunocompromised patient. N Engl J Med 2003; 348: 867-868.
9. Souza TM, Salluh JI, Bozza FA, et al. H1N1pdm influenza infection in hospitalized cancer patients: clinical evolution and viral analysis. PLoS One 2010; 5: e14158.
10. Cordero E, Aydillo T, Fariñas MC, et al. Novel Influenza A(H1N1) Study Group of the Spanish Network for Research in Infectious Diseases (REIPI). Immunosuppressed patients with pandemic influenza A 2009 (H1N1)

virus infection. Eur J Clin Microbiol Infect Dis 2009; 31: 547-556.
11. Chin-Yee BH, Monkman K, Hussain Z, et al. Attitudes toward vaccination for pandemic H1N1 and seasonal influenza in patients with hematologic malignancies. J Support Oncol 2011; 9: 156-160.
12. Monkman K, Mahony J, Lazo-Langner A, et al. The pandemic H1N1 influenza vaccine results in low rates of seroconversion for patients with hematological malignancies. Leuk Lymphoma 2011; 52: 1736-1741.
13. Mackay HJ, McGee J, Villa D, et al. Evaluation of pandemic H1N1 (2009) influenza vaccine in adults with solid tumor and hematological malignancies on active systemic treatment. J Clin Virol 2011; 50: 212-216.
14. Mariotti J, Spina F, Carniti C, et al. Long-term patterns of humoral and cellular response after vaccination against influenza A (H1N1) in patients with hematologic malignancies. Eur J Haematol 2012; 89: 111-119.

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