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CASE REPORT

Outbreak of pandemic 2009 influenza A/H1N1 infection in the hematology ward: fatal clinical outcome of hematopoietic stem cell transplant recipients and emergence of the H275Y neuraminidase mutation

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Abstract We report an outbreak of pandemic 2009 influenza A/H1N1 virus (2009 H1N1) infection that occurred in the hematology ward of our institution during the 2010–2011 influenza season. A total of seven hospitalized patients with hematologic tumors, including five recipients of hematopoietic stem cell transplantation (HSCT), successively developed rapid influenza detection test (RIDT)-positive influenza A; four patients had laboratory-confirmed 2009 H1N1 infection. Three HSCT recipients required mechanical ventilation support and two were admitted to the intensive care unit; they died of progressive respiratory failure despite receiving available anti-viral drugs. We implemented outbreak-control measures including transferal of RIDT-positive patients to a single-patient room and chemoprophylaxis with oseltamivir. We note that the H275Y neuraminidase mutation was detected in respiratory specimens from three patients, who were administered therapeutic or prophylactic dosages of oseltamivir. The present report demonstrates that the nosocomial 2009 H1N1 outbreak in the hematology ward led to fatal clinical outcomes and the emergence of a resistant virus at a markedly high rate.

Keywords Pandemic 2009 H1N1 influenza virus · Nosocomial outbreak · Hematopoietic stem cell transplantation · Outbreak-control measures · H275Y mutation

On behalf of the Hospital Infection Control Committee.

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Introduction

Pandemic 2009 influenza A/H1N1 virus (2009 H1N1) infection has been described to be associated more frequently with severe respiratory disease than seasonal influenza in recipients of hematopoietic stem cell transplant (HSCT) [1]. On the other hand, it has been shown that early initiation of treatment with oseltamivir for 2009 H1N1 influenza patients with cancer and/or HSCT recipients led to favorable clinical outcomes with no severe morbidity or influenza-related mortality, in spite of the high rate of hospitalization [2]. Nevertheless, this management for immunocompromised patients can enhance emergence of an oseltamivir-resistant virus having the H275Y neuraminidase mutation [3].

Here, we report an outbreak of 2009 H1N1 infection that occurred in the hematology ward of our institution during the 2010–2011 influenza season. The infection was initially recognized in a patient with relapsed multiple myeloma, who had received autologous HSCT. Shortly, thereafter, an additional 6 inpatients, including another 4 HSCT recipients, successively developed the infection and the 2009 H1N1 virus with the H275Y mutation was detected in 3 patients. In this report, we represent the clinical characteristics, response to treatment, and ultimate outcomes of

2009 H1N1 infection in a total of 7 patients as well as outbreak-control measures implemented by the Hospital Infection Control Committee.

Patients and methods

Table 1 summarizes clinical features of the 7 relevant patients with hematologic tumors. Of 5 HSCT recipients, 2 had undergone autologous HSCT, while 3 had received allogeneic HSCT grafts. Rituximab was included in the conditioning regimens for 3 patients whose tumor cells expressed CD20. Two patients had been admitted to the hospital due to a relapse of primary disease, while 4 HSCT recipients had been hospitalized for a long period for morbidities associated with HSCT, resulting in poor performance statuses (case 2 through 5). The remaining patient had refractory angioimmunoblastic T cell lymphoma (case 7). None of the patients had received the 2010–2011 vaccine protecting against the influenza A H3N2 virus, influenza B virus, or 2009 H1N1 virus.

Nasopharyngeal swab was collected within 48 h of the onset of symptoms. Influenza A infection was detected by a commercially available rapid influenza diagnostic test (RIDT; Quick Chaser® Flu A, B, MIZUHO MEDY Co. Ltd., Tosu). Selected specimens were sent to the Nara Prefectural Institute for Hygiene and Environment to obtain detailed information on the specific influenza A virus subtype as well as the presence or absence of the H275Y neuraminidase mutation; assays were performed according to the manual provided by the National Institute of Infectious Diseases. Respiratory specimens, including endotracheal aspirates of intubated patients, were repeatedly collected to assess the effectiveness of the treatment and to monitor emergence of the mutation.

Each patient had previously signed an informed consent form that allowed the use of clinical information for future research. This publication was approved by the Institutional Ethics Review Committee.

Results

Clinical symptoms at presentation

The 7 patients, who had been hospitalized in the hematology ward at the same time, successively developed RIDT-positive influenza A within 14 days and 4 patients had laboratory-confirmed 2009 H1N1 infection (cases 3, 4, 5, and 7), indicating a nosocomial 2009 H1N1 influenza outbreak. Presenting symptoms were a >38 °C fever in 6 patients and upper respiratory symptoms in 6. Five patients showed localized or diffuse pulmonary infiltrates on chest radiograph and 5 HSCT recipients required oxygen support and/or mechanical ventilation at the onset of illness. Laboratory data included white cell counts ranging from 300 to

Table 1 Baseline clinical characteristics of seven patients with hematologic tumors

Case no.	Age/sex	Primary tumor/ status	Hematopoietic stem cell transplantation (HSCT)				PS	Treatment
			Source	Conditioning	Days after HSCT	Comorbidities		
1	65/male	MM/relapse	Auto-PBSC	HDC	1,643	None	1	Bortezomib
2	56/female	ALL/remission	Allo-BM from MRD	RIC plus rituximab	308	Extensive cGVHD, hemorrhagic cystitis	2	Cyclosporine, prednisolone
3	65/male	ML/remission	Auto-PBSC	HDC plus rituximab	223	Chronic urinary tract infection	2	
4	67/female	ML/remission	Allo-BM from MUD	RIC plus rituximab	226	Extensive cGVHD	4	Tacrolimus, mycophenolate mofetil, prednisolone,
5	49/female	ALL/remission	Cord blood	MAC	1,315	Post-transplant lymphoproliferative disorder	4	Rituximab
6	67/male	ML/relapse	NA	NA	NA	NA	1	Salvage chemotherapy, rituximab
7	84/female	ML/refractory	NA	NA	NA	NA	3	Salvage chemotherapy

NA not applicable, *MM* multiple myeloma, *ALL* acute lymphocytic leukemia, *ML* malignant lymphoma, *Auto-PBSC* autologous peripheral blood stem cell, *allo-BM* allogeneic bone marrow, *MRD* HLA-matched related donor, *MUD* HLA-matched unrelated donor, *HDC* high-dose chemotherapy, *RIC* reduced-intensity conditioning regimen consisting of fludarabine, busulfan, and 4 Gy total body irradiation, *MAC* myeloablative conditioning regimen consisting of high-dose cyclophosphamide and 12 Gy total body irradiation, *cGVHD* chronic graft-versus-host disease, *PS* performance status

6,700/μl, neutrophils from 10 to 5,270/μl, and lymphocytes from 20 to 1,710/μl. Immunoglobulin G levels in the serum, except for case 1 with multiple myeloma, were from 420 to 1,316 mg/dl. Superimposed bacterial/fungal infection was recognized in 3 patients (Table 2).

Implementation of outbreak-control measures

The Hospital Infection Control Committee implemented outbreak-control measures, immediately after the first patient (case 1) was recognized as developing RIDT-positive influenza A. The hematology ward at that time admitted a total of 50 patients including 14 HSCT recipients (Fig. 1). Our policies included transferal of an RIDT-positive patient to a single-patient room with employment of droplet precautions and chemoprophylaxis with oseltamivir to all patients in the ward, irrespective of underlying conditions. All patients were informed of the development of the outbreak and gave their consent to our policies.

Visitors were screened for symptoms of acute respiratory illness and entry to the ward was restricted. We temporally declined admission of new patients. Seven days after the development RIDT-positive influenza A in the last patient (case 4), i.e. 29 days after recognition of influenza in the first patient, we finally withdrew these control measures.

Response to anti-viral treatment and clinical outcomes

Three patients, including 2 HSCT recipients, responded well to anti-viral treatments in addition to appropriate

Table 2 Symptoms and laboratory data of seven patients at the onset of influenza

Case no.	Fever >38 °C	Upper RTS	Lower RTS	Hypoxemia	White blood cells (/μl)	Neutrophils (/μl)	Lymphocytes (/μl)	Serum IgG (mg/dl)	Creatinine (mg/dl)	Co-infection of respiratory tract
1	+	+	+	+	6,600	5,270	980	756[a]	1	*S. pneumoniae*
2	+	+	−	+	1,400	970	130	946	1	ND
3	+	+	+	+	6,700	4,680	1,330	420	2.3	ND
4	−	+	+	+	2,600	700	1,710	764	0.4	*Aspergillus*
5	+	+	+	+	4,000	2,820	880	1,316	2.3	*Klebsiella*, MSSA
6	+	+	+	−	300	10	240	911	1.3	ND
7	+	−	−	−	400	320	20	668	0.3	ND

RTS respiratory tract symptoms, *MSSA* methicillin-sensitive *S. aureus*, *ND* not detected

[a] Including M-protein

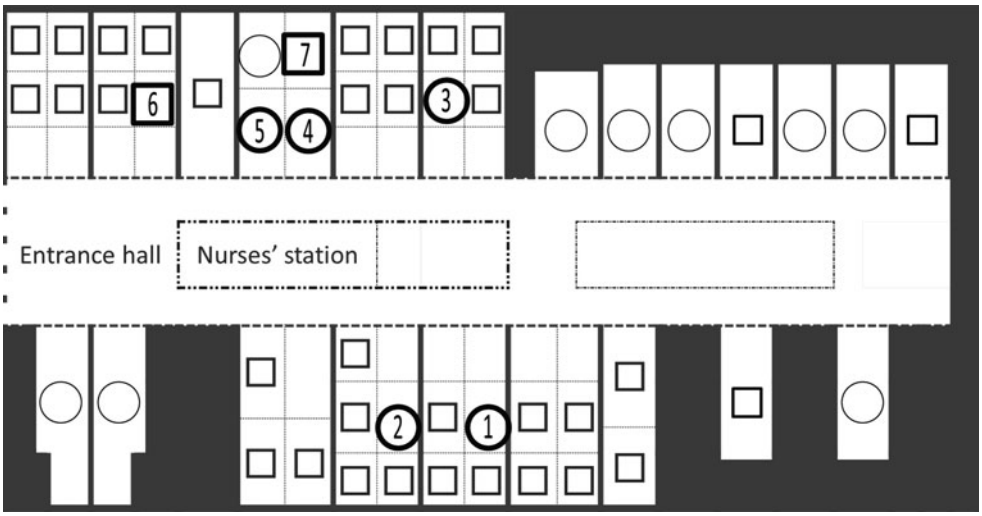

Fig. 1 Illustrative diagram of the hematology ward, consisting of 11 single-bed rooms, 10 multi-bed rooms, and 2 clean rooms for HSCT. Fourteen patients who underwent HSCT before or during the outbreak are indicated by *circles*. The 7 RIDT-positive patients, indicated by *numbers 1* through *7*, initially resided within the multi-bed rooms. Immediately after each patient was recognized to be RIDT-positive, 6 were transferred to a single-patient bed room within the ward and the remaining patient (case 5) was admitted to the intensive care unit in the independent ward, while case 4 was later transferred to the unit

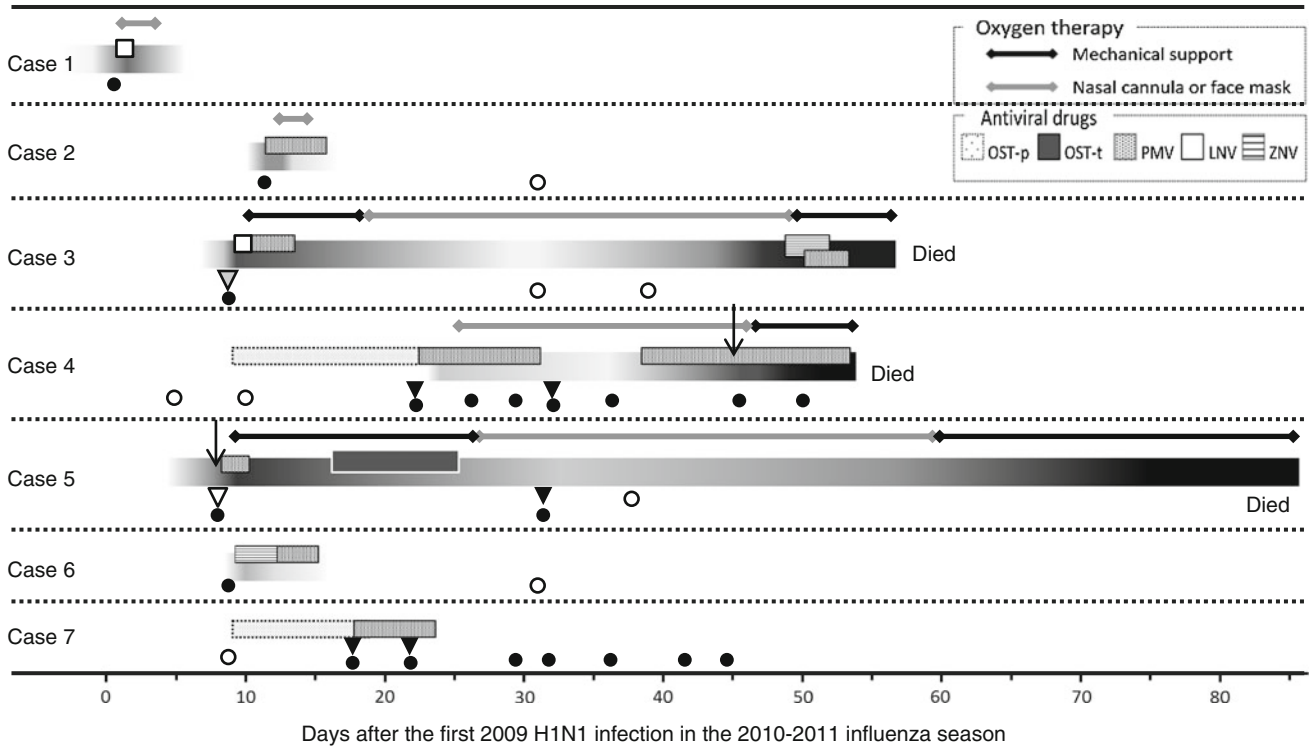

Fig. 2 Treatment and clinical course of 7 patients. *Closed* (positive) and *open* (negative) *circles* represent rapid diagnostic test of influenza A infection. 2009 H1N1 influenza infection determined by real-time reverse-transcriptase polymerase chain reaction is *indicated by triangles*; the presence (*closed triangle*) or absence (*open triangle*) of the H275Y mutation, resulting from the single nucleotide substitution, CAC to TAC, at position 275, was determined by sequencing the relevant region. Sequencing analysis of the specimen of case 3 was not performed (*gray triangle*). Anti-viral drugs were oseltamivir of prophylactic dose (OTV-p; 75 mg, orally, daily), oseltamivir of therapeutic dose (OTV-t; 75 mg, orally, twice daily), peramivir (PMV; 300 mg, intravenously, daily), laninamivir (LNV; 40 mg, single oral inhalation), and zanamivir (ZNV; 10 mg, oral inhalation, daily). The approximate severities of hypoxemia/respiratory failure of each patient are schematically represented by *gradated horizontal bars*. Cases 4 and 5 were admitted to the intensive care unit on the days indicated by *vertical arrows*

antibiotics (Fig. 2). In contrast, 3 HSCT recipients developed severe pneumonia requiring mechanical ventilation support and 2 (cases 4 and 5) were admitted to the intensive care unit (Fig. 2). They were treated with available anti-viral drugs, including intravenous peramivir and inhalation of zanamivir and laminamivir as well as high-dose methylprednisolone; each anti-viral drug was not necessarily administrated rationally, but was selected according to the clinical conditions of each patient (Fig. 2). In spite of these intensive treatments, these patients finally died of progressive respiratory failure with severe pneumonia and/or adult respiratory distress syndrome (ARDS) 32–82 days after the onset of influenza. Postmortem examination of the lungs of case 3 revealed diffuse alveolar damage with hyaline membrane formation; there was no evidence of bacterial/fungal co-infection.

Emergence of the H275Y mutant and prolonged virus shedding

The H275Y neuraminidase mutation was detected in respiratory specimens from 3 patients, who were administered therapeutic (75 mg, twice daily; case 5) or prophylactic dosages (75 mg, once daily; cases 4 and 7) of oseltamivir (Fig. 2). Case 4 became mutant-positive in association with worsening of respiratory distress and this positivity persisted until her death. Case 5 was initially infected with wild-type 2009 H1N1, while the second specimen had the mutation after 3 weeks. Case 7 showed prolonged shedding of the mutant virus over 19 days but lacked symptoms of influenza; the effect of the oseltamivir-resistant influenza virus in this patient remains to be determined.

Discussion

Most illnesses caused by the 2009 H1N1 virus were mild and self-limited [4], and the overall fatality rate in Japan was estimated to be as low as 0.15 per 100,000 [5]. In contrast, mortality rates associated with 2009 H1N1 infection in patients with hematologic tumors or HSCT recipients have been reported to be up to 46 % [6], even though these rates vary considerably among studies,

accounting for variable underlying conditions and settings [1–3, 6–8]. Our present report demonstrated that nosocomial 2009 H1N1 outbreak in the hematology ward can lead to fatal clinical outcome at a markedly high rate, i.e. 3 (43 %) of 7 patients with hematologic tumors and 3 (60 %) of 5 HSCT recipients. In one report, nosocomial acquisition of the virus itself was recognized as a risk factor requiring admission to the intensive care unit for mechanical ventilation [3]. Therefore, it is desirable for all healthcare workers in hematology wards to implement stringent infection control measures during an influenza season. On the other hand, risk factors identified by the data collection from the European Group for Blood and Marrow Transplantation and the Spanish Group of haematopoietic stem cell transplantation to be associated with significant morbidity and mortality in HSCT recipients who developed 2009 H1N1 infection, including age, lymphopenia (<300/μl), and neutropenia (<500/μl) [9], were not necessarily applicable to our cases (Tables 1, 2).

Pathogenesis of pneumonia/respiratory failure associated with 2009 H1N1 infection has not fully been established [4]. Viral RNA may be detected in secretions from the lower respiratory tract up to 28 days after the onset of severe pneumonia and longer in patients with immunosuppression [4]. In contrast, respiratory specimens from cases 3 and 5 of our cohort became negative for RIDT 22 and 29 days after the onset of influenza, while their pulmonary diseases rapidly deteriorated, suggesting that the virus alone may not have been responsible for the progression of respiratory failure or development of ARDS. A previous study has suggested the involvement of host factors, where patients who died or who had ARDS showed higher plasma levels of pro-inflammatory cytokines and chemokines than those in patients with mild disease [10].

It is noteworthy that the H275Y mutant virus was detected in 3 of 7 patients. Since case 5 was initially infected with the wild-type virus and was immediately admitted to the intensive care unit localized in the independent ward (Figs. 1, 2), and since the other two patients were each isolated in a single-patient room following infection-control measures, it is unlikely that the mutant virus was transmitted among these 3 patients, but rather H275Y mutants seem to have developed independently in each patient. The Infectious Disease Surveillance Center reported that oseltamivir-resistant 2009 H1N1 strains account for 1.0 % (79/8,145 in the 2009–2010 season) to 2.0 % (78/3,805 in the 2010–2011 season) of all isolated viruses in Japan [11]. On the other hand, the risk of the 2009 H1N1 virus developing the H275Y mutation that confers oseltamivir resistance is considered to be higher in immunocompromised patients [3]. Our present report suggests that universal chemoprophylaxis with oseltamivir for severely immunocompromised patients, such as HSCT recipients, during an outbreak can facilitate the development and/or selective growth of the oseltamivir-resistant virus. This is in clear contrast with immunocompetent hosts, in whom an oseltamivir "ring" prophylaxis was effective in reducing the impact of outbreaks of 2009 H1N1 in semi-closed environments without emergence of the H275Y strain [12].

Given that the response to the influenza vaccination is significantly reduced in immunocompromised patients [13], even though patients receiving the influenza vaccine 6 months or later after HSCT had a lower risk for virological confirmed influenza [14], anti-virals are central to the treatment of 2009 H1N1 infection. It is apparent that the standard dose and duration of oseltamivir, i.e. 75 mg twice daily for 5 days, are insufficient for immunocompromised patients. Instead, an increased dose and duration of oseltamivir, as well as combining this treatment with other available anti-viral drugs, may be initiated at the onset of symptoms of influenza irrespective of the severity of the illness. Prospective randomized studies for patients with hematologic tumors or HSCT recipients are needed to determine the optimal dose and schedule of anti-virals to efficiently eradicate the 2009 H1N1 virus from these very vulnerable patients.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Choi SM, Boudreault AA, Xie H, Englund JA, Corey L, Boeckh M. Differences in clinical outcomes after 2009 influenza A/H1N1 and seasonal influenza among hematopoietic cell transplant recipients. Blood. 2011;117:5050–6.
2. Redelman-Sidi G, Sepkowitz KA, Huang CK, Park S, Stiles J, Eagan J, et al. 2009 H1N1 influenza infection in cancer patients and hematopoietic stem cell transplant recipients. J Infect. 2010; 60:257–63.
3. Tramontana AR, George B, Hurt AC, Doyle JS, Langan K, Reid AB, et al. Oseltamivir resistance in adult oncology and hematology patients infected with pandemic (H1N1) 2009 virus. Aust Emerg Infect Dis. 2010;16:1068–75.
4. Bautista E, Chotpitayasunondh T, Gao Z, Harper SA, Shaw M, Uyeki TM, et al. Clinical aspects of pandemic 2009 influenza A (H1N1) virus infection. N Engl J Med. 2010;362:1708–19.
5. Infectious Agents Surveillance Center. 2009/2010 influenza season. Jpn IASR. 2010;31:248–50.
6. Wei JY, Chen FF, Jin J, Mai WY, Qian WB, Meng HT, et al. Novel influenza A (H1N1) in patients with hematologic disease. Leuk Lymphoma. 2010;51:2079–83.
7. Lalayanni C, Sirigou A, Iskas M, Smias C, Sakellari I, Anagnostopoulos A. Outbreak of novel influenza A (H1N1) in an adult

haematology department and haematopoietic cell transplantation unit: clinical presentation and outcome. J Infect. 2010;61:270–2.
8. Rihani R, Hayajneh W, Sultan I, Ghatasheh L, Abdel-Rahman F, Hussein N, et al. Infections with the 2009 H1N1 influenza virus among hematopoietic SCT recipients: a single center experience. Bone Marrow Transplant. 2011;46:1430–6.
9. Ljungman P, de la Camara R, Perez-Bercoff L, Abecasis M, Nieto Campuzano JB. Outcome of pandemic H1N1 infections in hematopoietic stem cell transplant recipients. Haematologica. 2011;96:1231–5.
10. To KK, Hung IF, Li IW, Lee KL, Koo CK, Yan WW, et al. Delayed clearance of viral load and marked cytokine activation in severe cases of pandemic H1N1 2009 influenza virus infection. Clin Infect Dis. 2010;50:850–9.
11. Infectious Agents Surveillance Center. 2010/2011 influenza season. Jpn IASR. 2011;32:314–6.
12. Lee VJ, Yap J, Cook AR, Chen MI, Tay JK, Tan BH, et al. Oseltamivir ring prophylaxis for containment of 2009 H1N1 influenza outbreaks. N Engl J Med. 2010;362:2166–74.
13. Issa NC, Marty FM, Gagne LS, Koo S, Verrill KA, Alyea EP, et al. Seroprotective titers against 2009 H1N1 influenza A virus after vaccination in allogeneic hematopoietic stem cell transplantation recipients. Biol Blood Marrow Transplant. 2011;17:434–8.
14. Ljungman P, Avetisyan G. Influenza vaccination in hematopoietic SCT recipients. Bone Marrow Transplant. 2008;42:637–41.