Table 1. General and health-related backgrounds of study subjects.

	Pandemic period*	Post-pandemic period*	Total	p value
	N = 211 (69.9%)	N = 91 (30.3%)	N = 302 (100.0%)	
Gender				
Male-no. (% of group)	128 (60.7)	50 (54.9%)	178 (58.9%)	0.354
Age – median (range)	38.5 (0–90)	42.0 (2–91)	39.0 (0–91)	0.003
Age group – no. (% of group)				0.001
<18	45 (21.4)	4 (4.4)	49 (16.3)	
18-<50	108 (51.2)	52 (57.1)	160 (53.0)	
50-<65	43 (20.4)	22 (24.2)	65 (21.5)	
≥65	15 (7.1)	13 (14.3)	28 (9.3)	
Education background – no. (% of group)				0.356
None	34 (16.4)	17 (17.9)	51 (16.9)	
Primary school	57 (27.5)	25 (26.3)	82 (27.2)	
Secondary school	49 (23.7)	16 (16.8)	65 (21.5)	
High school	33 (15.9)	19 (20.0)	52 (17.2)	
University	30 (14.5)	12 (12.6)	42 (13.9)	
Graduate school	2 (1.0)	3 (3.2)	5 (1.7)	
Technical school	2 (1.0)	3 (3.2)	5 (1.7)	
Occupation – no. (% of group)				0.437
Unemployed	18 (8.6)	17 (18.7)	35 (11.7)	
Retired	1 (0.5)	2 (2.2)	3 (1.0)	
Student	28 (13.4)	3 (3.3)	31 (10.3)	
Housewife	53 (25.4)	30 (33.0)	83 (27.7)	
Governmental employee	4 (1.9)	3 (3.3)	7 (2.3)	
Employee by private company	30 (14.4)	15 (16.5)	45 (15.0)	
Commercial	33 (15.8)	5 (5.5)	38 (12.7)	
Self-employed (small business)	28 (13.4)	3 (3.3)	31 (10.3)	
Socioeconomic level†				0.332
Low	144 (72.4)	69 (67.0)	213 (70.5)	
Middle	55 (27.6)	34 (33.0)	89 (29.5)	
High	0 (0.0)	0 (0.0)	0 (0.0)	
Heath-related background				
Vaccination (seasonal influenza)				0.203
Vaccinated in 2009	4 (1.9)	6 (6.6)	10 (3.3)	
Vaccinated in 2010	42 (19.9)	20 (22.0)	62 (20.5)	
Vaccinated in 2011	8 (3.8)	3 (3.5)	11 (3.6)	
Smoking				0.002
smoker	18 (8.5)	21 (23.1)	39 (12.9)	
ex-smoker	19 (9.0)	8 (8.8)	27 (8.9)	
Chronic respiratory illness on medication	6 (2.8)	40 (44.0)	46 (15.2)	0.000
Days from symptom onset to initiation of treatment – median (range)‡	6.0 (0–35)	5.0 (0–29)	6.0 (0–35)	0.379

*Pandemic period, between April 2009 and July 2010; Post-pandemic period, between August 2010 and the end of survey period.
†Socioeconomic level evaluated by ability to pay for utilities, food, and medical service: low income, cannot cover electricity, water, telephone, house rent, foods, any medical service; middle income, can cover a part of electricity, water, telephone, house rent, and foods, but not any medical service; high income, can cover utility and adequate goods, and medical services.
‡Number of days from symptom onset to initiation of oseltamivir administration.
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care services (consultation, hospitalization, and medication) or some of them (consultation and hospitalization) were 28.8% and 67.6%, respectively, in Group-pdm vs. 17.8% and 38.9%, respectively, in Group-post ($p<0.001$); 82.8% of subjects were uninsured.

Table 2. Detailed economic status of study subjects.

	Pandemic period*i	Post-pandemic period*	Total	p value
	N = 211 (69.9%)	N = 91 (30.3%)	N = 302 (100.0%)	
Income ability to pay for utility services†				0.000
All services	76 (36.0)	16 (17.6)	92 (30.5)	
2–3 of all	133 (63.0)	41 (45.1)	174 (57.6)	
None	2 (0.9)	34 (37.4)	36 (11.9)	
Income ability to pay for food‡				0.000
All necessaries	126 (59.7)	25 (27.8)	151 (50.2)	
2–3 of all	85 (40.3)	51 (56.7)	136 (45.2)	
None	0 (0.0)	14 (15.6)	14 (4.7)	
Income ability to pay for Health care service				0.000
Consultation, hospitalization, medication	60 (28.6)	16 (17.8)	76 (25.3)	
Consultation, hospitalization	142 (67.6)	35 (38.9)	177 (59.0)	
None	8 (3.8)	39 (43.3)	47 (15.7)	
Health insurance				0.144
None	179 (84.8)	71 (78.0)	250 (82.8)	
Government insurance§	17 (8.1)	8 (8.8)	25 (8.3)	
Private insurance	1 (0.5)	0 (0.0)	1 (0.3)	
Others	14 (6.6)	12 (13.2)	26 (8.6)	

*Pandemic period, between April 2009 and July 2010; Post-pandemic period, between August 2010 and the end of survey period.
†Income can pay for expense of utilities; light, gas, water, sewerage, telephone.
‡Income can pay for expense of food; meat, egg, milk, cereals, vegetable.
§Governmental insurance included workers in private organizations.
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Life environment

Most of the subjects in Group-pdm and Group-post lived in an area that provided all public services ($p = 0.144$), and there was no difference between the groups. In terms of housing status (Table 3), 39.8% and 30.8% of subjects in Group-pdm and Group-post lived for free in borrowed accommodation, whereas 18.5% and 34.1%, respectively, lived in rental accommodation; there was a significant difference in housing status between the groups ($p = 0.011$). Although most Group-pdm subjects lived in concrete houses or a combination of concrete and tinplate houses, more subjects in Group-post lived only in tinplate houses, and there was a significant difference in housing quality between the groups ($p<0.001$). The number of rooms and individuals living in a house were significantly different between the groups ($p = 0.003$ and $p<0.001$, respectively). The median number of individuals per room in each household was 2.5 (range, 0.29–8.0) in Group-pdm and 3.0 (range, 0.67–9.0) in Group-post, and there was a significant difference between the groups ($p<0.001$).

Information relating to H1N1 virus infection

The most common source of information about influenza A(H1N1)pdm09 was television in both groups ($p = 0.706$) (Table 4). Although subjects in Group-pdm were more likely than those in Group-post to be informed through radio (55.5% vs. 25.3%, $p<0.001$), there was no significant difference in the use of other sources of information between the groups.

More Group-pdm subjects than Group-post subjects received clear information about methods of prevention of influenza A(H1N1)pdm09 (77.3% vs. 29.7%, respectively, $p<0.001$) as well as information regarding the necessity for early access to health care (94.3% vs. 60.4%, respectively, $p<0.001$).

Delay in seeking health care from symptom onset in hospitalized patients with pneumonia

In the multivariate regression analysis, the number of household rooms, information regarding the necessity for quick access to health care, and housing construction materials were independent factors that tended to be associated with the number of days from symptom onset to the initiation of antiviral treatment (Table 5). Since the INER administered antiviral treatment soon after hospital admission to hospitalized patients with pneumonia, the number of days to initiation of antiviral treatment was practically the same as the number of days from symptom onset to first access to formal health care.

Discussion

Low awareness of the importance of early access to healthcare and difficulty separating oneself from other individuals in a household owing to poverty are possible reasons for hospitalized pneumonia due to influenza virus infection in the post pandemic period.

INER is a tertiary medical organization for the care of patients with respiratory illness, and it provides medical services mainly to uninsured individuals in the metropolitan area of Mexico City and neighboring states. Most patients who visit the INER have a similar low socioeconomic level, demographic characteristics, and educational background [17], including the subjects in the present

Table 3. Life environmental qualities of study subjects.

	Pandemic period*	Post-pandemic period*	Total	p value
	N = 211 (69.9%)	N = 91 (30.3%)	N = 302 (100.0%)	
Location†				0.144
All public services	187 (88.6)	75 (82.4)	262 (86.8)	
Partial public services	24 (11.4)	16 (17.6)	40 (13.2)	
Housing				0.011
Borrow without any payment	84 (39.8)	28 (30.8)	112 (37.1)	
Rent	39 (18.5)	31 (34.1)	70 (23.2)	
Pay for credit	16 (7.6)	2 (2.2)	18 (6.0)	
Own	72 (34.1)	30 (33.0)	102 (33.8)	
House construction material				<0.001
Concrete	179 (84.8)	70 (76.9)	249 (82.5)	
Tinplate	3 (1.4)	12 (13.2)	15 (5.0)	
Concrete and tinplate	29 (13.7)	9 (9.9)	38 (12.6)	
Number of rooms in a house				0.003
≤2	85 (40.3)	64 (70.3)	149 (49.3)	
3–5	121 (57.3)	26 (28.6)	147 (48.7)	
≥6	5 (2.4)	1 (1.1)	6 (2.0)	
Number of individuals in a house				<0.001
≤2	13 (6.2)	24 (26.4)	37 (12.3)	
3–5	167 (79.5)	57 (62.6)	224 (74.4)	
≥6	30 (14.3)	10 (11.0)	40 (13.3)	
Number of individuals per a room, mean (range)	2.5 (0.29–8.0)	3.0 (0.67–9.0)	2.5 (0.29–9.0)	<0.001

*Pandemic period, between April 2009 and July 2010; Post-pandemic period, between August 2010 and the end of survey period.
†Location was defined by the accessibility of public service which is also followed by the Social Gap Index.
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study (Table 1). A detailed evaluation of socioeconomic similar subjects using the Social Gap Index of CONEVAL [16] revealed

Table 4. Availability of information related to influenza A(H1N1)pdm09.

	Pandemic period*	Post-pandemic period*	Total	p value
	N = 211 (69.9%)	N = 91 (30.3%)	N = 302 (100.0%)	
Information resources				
Newspaper	34 (16.1)	13 (14.3)	47 (15.6)	0.688
Television	170 (80.6)	75 (82.4)	245 (81.1)	0.706
Radio	116 (55.5)	23 (25.3)	139 (46.3)	0.000
Internet	4 (1.9)	3 (3.3)	7 (2.3)	0.355
Family and friends	22 (10.4)	15 (16.5)	37 (12.3)	0.141
Healthcare workers in Hospital	23 (10.9)	3 (3.3)	26 (8.6)	0.031
No information	1 (0.5)	3 (3.3)	4 (1.3)	0.083
Received clear information how to prevent influenza?	163 (77.3)	27 (29.7)	190 (62.9)	0.000
Received the information for the necessity of quick access to healthcare.	199 (94.3)	55 (60.4)	254 (84.1)	0.000

*Pandemic period, between April 2009 and July 2010; Post-pandemic period, between August 2010 and the end of survey period.
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Table 5. Factors relating to delayed seeking of health care, using multivariate analysis.

	Coefficient	Standard error	t value	p value	95% confidence interval
Constant	−10.246	4.351	−2.355	0.022	−18.985–−1.508
Number of rooms in house	3.798	0.895	4.242	0.000	2.000–5.597
Received information about necessity of quick access to health care during pandemic period	4.741	1.986	2.387	0.021	0.751–8.730
House construction material*	3.056	1.473	2.075	0.043	0.097–6.015

*House constructed of concrete, tinplate, and combination of concrete and tinplate.
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that <1% in Group-pdm were unable to pay for either utility services or food; there was a greater number of such subjects in Group-post (Table 2). Moreover, approximately half of the subjects in Group-post were unable to pay for health-care services. In contrast to Group-pdm subjects, those in Group-post lived in houses constructed of tinplate with fewer rooms, and there was also a greater number of individuals sharing the same house (Table 3). These results reflect the situation of many poor people in Mexico City, who live together with relatives and friends and help one another in their daily lives, including payments for utilities and food [19]. The subjects in Group-post were more likely to be impoverished than those in Group-pdm and showed a greater tendency to engage in mutual support. Economic difficulties and the inability to pay for treatment create problems in accessing formal health care in the early stage of any illness, even if the cause is acute viral infection; these factors can lead to the delayed initiation of appropriate treatment. For people without health-care insurance and who are paid on a daily basis, it is especially hard to stop work and seek medical assistance, even for a day. As a result, by the time they present to a hospital, their disease has progressed and may have become severe. This was the situation for patients in both Group-pdm and Group-post; however, those in Group-post faced greater poverty. There was a greater number of patients facing economic difficulties in Group-post than in Group-pdm, which indicates that subjects in the former group may have experienced more problems in accessing early health care. We previously showed that patients with severe pneumonia had a lower socioeconomic level and delayed initiation of oseltamivir treatment [14]. Patients in Group-post lived in houses with fewer rooms, but they also lived together with a greater number of other individuals (Table 3). This reflects not only the socioeconomic level of the subjects, but also an increased risk for human-to-human transmission of the influenza virus.

In Mexico, rural poverty is concentrated in southern areas of the country [19,20]. Especially during the early stage of the influenza outbreak in 2009, there was a high rate of infection in populations in areas of rural poverty in the south including Mexico City [21]. However, Mexico City is not a single metropolitan area but a growing megalopolis. The city incorporates surrounding areas of poverty, and low- and middle-income communities live in close proximity in the same area. Most of the subjects in the present study were impoverished; however, >80% of them were located in areas with access to all public services, and there was no significant difference between the subjects in Group-pdm and Group-post (Table 3). This is typical of the unique living environment in Mexico City, and it reflects the traditional Mexican custom of social support, whereby high- and middle-income individuals help those with low or no income [19]. Impoverished people in Mexico City depend for their daily existence on those with high and middle incomes; therefore, they need to live close to high- and middle-income areas. As a result, there was no significant difference in residential location between the subjects in Group-pdm and Group-post (Table 3).

Seasonal influenza vaccination in Mexico is limited to the young and elderly [22]. Although a previous study reported that vaccination status was independently associated with H1N1 influenza [23], there was no significant difference between the groups in the present study (Table 1). Although smoking is also associated with H1N1 influenza [2,18], there were significantly more smokers in Group-post than in Group-pdm ($p = 0.002$), which may reflect the fact that there were more elderly patients in Group-post ($p = 0.001$). In terms of comorbid conditions, more patients in Group-post had chronic respiratory illness than did those in Group-pdm ($p < 0.001$). These results indicate that H1N1 influenza is an emerging infectious disease that could infect individuals beyond the population without underlying respiratory illness. One year after the influenza outbreak, after some of the population had gained immunity [24,25], the elderly population with underlying respiratory illness and who were smokers were more likely to be susceptible to influenza virus infection than the younger population without underlying respiratory illness.

The time from onset of symptoms to initiation of oseltamivir treatment is a key factor in reducing severe respiratory conditions due to H1N1 influenza [14,15]. The time to initiation of oseltamivir treatment depended on health-care-seeking behavior. After the first manifestation of the outbreak in Mexico, the mass media drew attention to the disease and created a sense of fear in the population [18]. However, among impoverished individuals and those with less education, it may be difficult to obtain information from media sources. Although television was a major source of information for the patients in our study (Table 4), more patients in Group-post than in Group-pdm did not receive information about methods of prevention of H1N1 influenza infection and the necessity for quick access to health care ($p<0.001$). This indicates the importance of the method of information distribution and education for enhancing the social response to an influenza pandemic. We also evaluated the factors affecting the time from symptom onset to initiation of oseltamivir treatment (Table 5). The number of rooms in the household, receiving information about the necessity of quick access to health care, and house construction materials were evaluated as independent factors that possibly influenced health-care-seeking behavior. Poverty is associated with difficult housing conditions including the number of rooms and house construction materials. It also associated with lower access to information from media resources that could motivate people to seek early access to health care owing to a lack of utility services in the household. In addition, fewer rooms in a household was associated with

increased risk of human-to-human infection. This indicates that poverty strongly influences health-care-seeking behavior and suggests the importance of distribution of information and educational resources.

The present study was limited to a population that was mostly uninsured and facing socioeconomic difficulties in Mexico City. Although there is a large gap between poverty and wealth in Mexico, the present study did not evaluate the range of socioeconomic levels in the population. Patients in Group-pdm had H1N1 influenza confirmed by RT-PCR, but the same test was not performed in patients in Group-post for budgetary reasons in the INER. Therefore, Group-post may have included patients with pneumonia not caused by influenza A(H1N1)pdm09 virus, but by some other type of influenza A virus. Further study, including an investigation of different socioeconomic populations, is needed to determine the impact of socioeconomics on the severity of disease due to influenza infection.

Although many factors affect disease occurrence and severity (including pneumonia), health-care-seeking behavior, poverty, and distribution of information are important factors from a socioeconomic point of view. These factors may explain the different patterns of morbidity and mortality for influenza A(H1N1)pdm09 in different countries and regions.

Author Contributions

Conceived and designed the experiments: TM ALHI KK. Performed the experiments: ALHI MEVM TM KK JT SI ELMV LAR. Analyzed the data: TM ALHI KK. Wrote the paper: TM KK.

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ORIGINAL ARTICLE

A national survey on myocarditis associated with influenza H1N1pdm2009 in the pandemic and postpandemic season in Japan

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Abstract An influenza pandemic occurred in 2009. We performed a retrospective national questionnaire survey about H1N1pdm2009 myocarditis to compare influenza A H1N1pdm2009 myocarditis in the pandemic (2009/2010) and postpandemic seasons (2010/2011) by collecting data from 360 hospitals. The diagnosis of myocarditis was performed using the guidelines for Diagnosis and Treatment of Myocarditis published by the Japanese Circulation Society (JCS 2009). Twenty-nine patients with influenza A H1N1pdm2009 myocarditis were reported, with 25 from the 2009/2010 season and only 4 patients from the 2010/2011 season. Morbidity and mortality was 28 % (8/29) among all the myocarditis patients. Six patients with myocarditis were complicated by pneumonia. Myocarditis was proved by endomyocardial biopsy or autopsy in 9 patients, although histological findings showed mild myocarditis even in clinically defined fulminant myocarditis cases. Seventeen patients were diagnosed with fulminant H1N1pdm2009 myocarditis with fatal arrhythmias or varying degrees of cardiogenic shock. Fifteen fulminant myocarditis patients were seen in the 2009/2010 season and only 2 in the 2010/2011 season. Ventilators were used in 16 patients. Mechanical circulatory support with intraaortic balloon pumping or percutaneous cardiopulmonary support (IABP/PCPS) was emergently inserted in 13 patients. Of these, 9 patients were rescued with mechanical circulatory support, and 4 patients died. Four fulminant myocarditis patients treated without IABP/PCPS died. We described the clinical features of patients with myocarditis associated with influenza H1N1pdm2009 in the pandemic and postpandemic seasons and demonstrated the high prevalence of fulminant myocarditis (17/29, 59 %). The number of patients with myocarditis associated with influenza A virus seemed to increase in the pandemic season.

Keywords Myocarditis · Influenza A · Pandemic

Introduction

Acute myocarditis is a potentially lethal disease: the etiological agents of viral myocarditis include enteroviruses, adenoviruses, parvoviruses, cytomegalovirus, and influenza viruses [1–7]. Fulminant myocarditis causes severe hemodynamic dysfunction that requires high-dose catecholamine and mechanical circulatory support [1, 7]. Although the frequency of myocardial involvement in influenza infection is variable, that of fulminant myocarditis associated with influenza infection is rare, as shown in previous papers [1–4]. An influenza pandemic occurred in 2009 [8–12]. The Ministry of Health, Labor and Welfare of Japan (MHLW) confirmed only 198 deaths among about 20.61 million patients infected with influenza A H1N1pdm2009 in the 2009/2010 season and 150 deaths among about 10.3 million patients in the 2010/2011 season in Japan [10, 11]. Although usually

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both the diagnosis and treatment of the pathogen involved in myocarditis are difficult, in the 2009/2010 pandemic, adequate diagnostic methods, such as the rapid influenza tests and reverse transcription-polymerase chain reaction (RT-PCR) for influenza H1N1pdm2009, and treatment with neuraminidase inhibitors were already available [10–13]. By conducting a cross-sectional national survey with assistance from all members of the Japanese Circulation Society (JCS) in the 2009/2010 influenza season, we previously reported 15 H1N1pdm2009 myocarditis patients and demonstrated their clinical features [13]. The Japanese Circulation Society performed a prospective study of patients with myocarditis associated with H1N1pdm2009 in the 2010/2011 season using their website, although no case was reported. Therefore, to compare myocarditis associated with influenza H1N1pdm2009 in the pandemic (2009/2010) and post-pandemic (2010/2011) seasons, we performed a national survey with a fill-in-the-blanks and multiple-choice questionnaire mailed to hospitals in Japan that have cardiology or pediatric departments or both.

Patients and methods

We mailed questionnaires to 978 hospitals in Japan that have cardiology and pediatric departments to compare myocarditis associated with influenza H1N1pdm2009 in the pandemic (2009/2010) and postpandemic (2010/2011) seasons. A fill-in-the-blanks and multiple-choice questionnaire was designed to obtain information on patient profiles (sex, age, and baseline disease), symptoms of influenza, symptoms of myocarditis, laboratory findings (e.g., cardiac enzymes, ECG, echocardiogram), treatment (e.g., neuraminidase inhibitors, steroid, mechanical circulatory support, ventilator), outcomes, and other. The questionnaire also included information about the number of hospitalizations associated with H1N1pdm2009 influenza during the two seasons. Myocarditis was diagnosed using the Guidelines for Diagnosis and Treatment of Myocarditis (JCS 2009) [1]. Compatible clinical symptoms, echocardiographic abnormalities in the absence of cardiac ischemia, and leakage of cardiac enzymes or other evidence of myocardial damage aided the diagnosis of myocarditis. Laboratory diagnosis of influenza A H1N1pdm2009 was made by quick influenza diagnostic testing or probe-based RT-PCR using a nasopharyngeal swab or sputum. The study protocol was approved by the Institutional Review Board of Osaka Medical College.

Results

We received completed questionnaires from 360 hospitals. Although 25 patients with myocarditis associated with influenza H1N1pdm2009 (17 men and 8 women; mean age, 39 ± 21 years) were reported to the task force of the Clinical Research for Myocarditis in the pandemic season (2009/2010), only 4 patients (3 men and 1 woman; mean age, 45 ± 15 years) were reported in the postpandemic season (2010/2011). Total mortality rate among all the myocarditis patients in both seasons was 28 % (8/29). Patient profiles, laboratory findings, treatments, and outcomes of patients with myocarditis associated with H1N1pdm2009 are shown in Table 1.

Myocarditis was proved by endomyocardial biopsy or autopsy in 9 patients (31 %); it was clinically diagnosed based on clinical features, leakage of cardiac constitutional proteins, such as troponin T/I, abnormalities on echocardiography, and other findings in the other 20 patients. Cardiac symptoms such as dyspnea, chest discomfort, hypotension, and syncope developed within 3 days of sickness in 16 patients (64 %). The most frequent baseline disease was a respiratory disorder in 7 (24 %) patients, including bronchial asthma in 5 patients (17 %) and emphysema in 2 patients (8 %). Six patients (21 %) with myocarditis were complicated by pneumonia. RT-PCR or quick diagnostic testing yielded positive results in all patients (100 %). Most patients exhibited ECG abnormalities, such as ST-T abnormalities (64 %). Echocardiography revealed abnormalities of left ventricular wall motion in 24 patients (83 %). Seven of the 9 patients of histologically proven myocarditis were fulminant myocarditis patients; 2 had acute myocarditis. Histological findings of these 9 patients showed myocarditis with lymphocyte infiltration. Quantitative assessment of troponin T/I was performed in 5 patients, in all of whom (100 %) it was elevated. On the other hand, qualitative quick troponin testing, which was conducted in 6 patients, was positive in only 3 patients (50 %). Cardiovascular magnetic resonance imaging (MRI) was performed in 2 patients. T_2-weighted cardiovascular MRI showed high-density signals in the region of the left ventricle in a 28-year-old man with fulminant myocarditis; his serial biopsies showed mild inflammation and degeneration of myocytes. RT-PCR testing for H1N1pdm2009 from heart specimens was performed in 2 cases (8 %), in both of whom it was negative. Cardiac dysfunction almost completely resolved in 19 patients (66 %) but remained partly unresolved in 2 patients (8 %). Coronary studies, which were performed in 20 patients (69 %), yielded normal results in all. Twenty-eight patients (96 %) were treated with neuraminidase inhibitors.

Seventeen of the 29 patients (59 %) were diagnosed with fulminant myocarditis with fatal arrhythmias and/or varying degrees of cardiogenic shock. Fifteen fulminant myocarditis patients (15/25, 60 %) were seen in the 2009/2010 season and only 2 (2/4, 50 %) in the 2010/2011 season. The clinical data of these 17 fulminant myocarditis patients are shown in Table 2. Nine (53 %) of the 17 fulminant myocarditis

Table 1 Patient profiles, laboratory findings, results of endomyocardial biopsies and/or autopsies, treatments, and outcomes of patients with myocarditis associated with H1N1pdm2009 in the 2009/2010 and 2010/2011 influenza seasons in Japan

	2009/2010 season	2010/2011 season
Number of H1N1pdm myocarditis cases	25	4
Sex (male/female)	17 (68 %)/8 (32 %)	1 (25 %)/3 (75 %)
Age (mean ± SD)	39 ± 21	45 ± 15
Survival	19 (76 %)	2 (50 %)
Adult/children	20 (80 %)/5 (20 %)	4 (100 %)/0 (0 %)
Fulminant myocarditis (adult/child)	15 (60 %) (11/4)	2 (50 %) (2/0)
Biopsy/autopsy	10 (40 %)/3 (12 %)	0/1 (25 %)
Baseline disease	Asthma, emphysema 6 (24 %)	Asthma 1 (25 %)
	DM 2 (8 %)	Hypertension 1 (25 %)
	None 14 (56 %)	None 1 (25 %)
Pneumonia	Viral 2 (8 %)/bacterial 2 (8 %)	Viral 1 (25 %)/bacterial 1 (25 %)
Cardiac symptoms	Dyspnea 13 (52 %)	Dyspnea 3 (75 %)
	Chest pain 4 (16 %)	Shock 1 (25 %)
	Syncope 3 (12 %)	Cyanosis 1 (25 %)
Onset of cardiac symptoms	1st–3rd day of sickness 16 (64 %)	1st–3rd day of sickness 0 (0 %)
	4th–10th day of sickness 6 (24 %)	4th–10th day of sickness 4 (100 %)
	Over 11th day of sickness 3 (12 %)	Over 11th day of sickness 0 (0 %)
ECG abnormalities	ST-T abnormalities 16 (64 %)	PSVT 2 (50 %)
	VT, VF 5 (20 %)	WNL 1 (25 %)
	Complete AV block 3 (12 %)	
	No information 2 (8 %)	No information 1 (25 %)
Echocardiogram	Wall motion abnormalities 21 (84 %)	Wall motion abnormalities 3 (75 %)
	Pericardial effusion 3 (12 %)	Pericardial effusion 1 (25 %)
	No information 2 (8 %)	No information 1 (25 %)
Peak CPK values	13,639 ± 42,495 IU/l	14,604 ± 18,770 IU/l
Coronary artery (CAG/CT)	No stenosis 15 (60 %)	No stenosis 3 (75 %)
	Not examined 10 (40 %)	Not examined 1 (25 %)
Other diagnostic tool (positive/examined)	Qualitative troponin 3/6 (50 %)	Qualitative troponin 0/0
	Quantitative troponin T/I 5/5 (100 %)	Quantitative troponin T/I 0/0
	MRI 1/2 (50 %)	MRI 0/0
Antiviral drug	24 (96 %)	4 (100 %)
Other treatment	Steroid 5 (20 %)	Steroid 0 (0 %)
	γ-Globulin 6 (24 %)	γ-Globulin 0 (0 %)

patients had no baseline disease. Three fulminant myocarditis patients (17 %) were complicated by pneumonia. Myocarditis was proved by endomyocardial biopsy or autopsy in 7 (41 %) of the 17 fulminant myocarditis patients. Histological findings were classified by the Dallas Criteria [14]. The first biopsy, obtained from a 44-year-old woman on day 1 showed myocarditis with lymphocytic infiltration, degeneration of myocytes, and interstitial edema; the second biopsy on day 23 showed resolving myocarditis. Histological findings in the other 8 patients showed myocarditis with infiltration of lymphocytes (ranging from mild to moderate, but not severe). Autopsy of the patient with fulminant myocarditis (on day 9) showed only interstitial fibrosis without lymphocytic infiltration. Ventilators were used in 16 patients (94 %). Mechanical circulatory support with intraaortic balloon pumping (IABP) and/or percutaneous cardiopulmonary support (PCPS) was emergently inserted in 13 patients. Nine of

Table 2 Patient profiles, laboratory findings, reports of endomyocardial biopsies and/or autopsies, treatments, and outcomes of patients with fulminant myocarditis associated with influenza A H1N1pdm2009 in Japan

	Fulminant myocarditis
Number of cases (adult/children)	17 (13/4)
Sex (male/female)	10 (58 %)/7(42 %)
Age (mean ± SD)	32 ± 19
Survival	9/17 (53 %)
Baseline disease	Asthma, emphysema 4 (24 %)
	DM 2 (12 %)
	None 9 (53 %)
Complicated pneumonia	3 (18 %)
Histological findings of heart tissue by Dallas criteria (1987) [14]	Resolving myocarditis (1st biopsy on day 1: myocarditis, LI, IE, MD; 2nd biopsy on day 23: LI)
	Myocarditis on day 1 (moderate LI, IE, MD)
	Myocarditis on day 1 (moderate LI, IE, MD)
	Myocarditis on day 4 (mild LI, IE, MD)
	Myocarditis on day 32 (mild LI, IE, MD)
	Borderline myocarditis (LI)
	Borderline myocarditis on day 7 (LI)
	No myocarditis on day 9 (IF)
	No myocarditis on day 16 (IF)
RT-PCR from heart tissue	0/2 (0 %) (on days 8 and 9)
Peak CPK values	23,640 ± 52,471 IU/l
Other diagnostic tool (positive/examined)	Qualitative troponin 2/3 (67 %)
	Quantitative troponin T/I 5/5 (100 %)
	MRI 1/2 (50 %)
Ventilator	15 (88 %)
IABP/PCPS	13 (76 %)
Survival with IABP/PCPS	9/13 (69 %)
Antiviral drug	16 (96 %)

Histological findings were classified by the Dallas Criteria [14]. First biopsy: myocarditis, borderline myocarditis, no myocarditis. Subsequent biopsies: ongoing myocarditis, resolving myocarditis, resolved myocarditis

LI lymph node infiltration, *IE* interstitial edema, *DM* diabetes mellitus, *MD* degeneration of myocyte, *IF* interstitial fibrosis

these 13 patients (69 %) were successfully rescued with mechanical circulatory support; the remaining 4 patients died (31 %). Four fulminant myocarditis patients treated without IABP/PCPS also died (100 %).

Discussion

The MHLW of Japan confirmed only 198 deaths (9.6×10^{-4} %) among about 20.61 million patients infected with influenza A H1N1pdm2009 in the 2009/2010 season, and 150 deaths (15×10^{-4} %) among about 10.3 million patients in the 2010/2011 season in Japan [10, 11]. The low case-fatality rate in Japan may be a result of early diagnosis and aggressive early intervention with antiviral drugs [9–11]. Twenty-five influenza H1N1pdm2009 myocarditis patients ($\geq 0.20 \times 10^{-4}$ %) were reported in the 2009/2010 season, although only 4 patients ($\geq 0.031 \times 10^{-4}$ %) were documented in the 2010/2011 season in the present study. The number of patients with clinically defined myocarditis associated with the influenza A virus seemed to increase in the pandemic season and obviously decrease in the postpandemic season compared to the pandemic season [2–4, 12, 13, 15–17]. The mean age (39 years) of myocarditis patients associated with H1N1pdm2009 influenza seemed to be lower than the age of patients with serious illness associated with seasonal influenza in the present study, probably indicating an age shift to a younger population in myocarditis patients with high fatality.

The frequency of myocardial involvement in influenza infection is variable, with fulminant myocarditis associated with seasonal influenza infection being rare, as shown in previous papers, probably because of the low affinity of the influenza virus for the myocardium [1–6]. Small autopsy-based studies demonstrated the complication rate of focal to diffuse myocarditis in fatal cases as 39 % with the 1957 Asian influenza pandemic and 48 % with the Spanish influenza pandemic [2]. Myocarditis caused by influenza is likely to be a terminal event in patients during influenza pandemics. In our survey, a total of 17 fulminant myocarditis patients were reported, 8 of whom died (47 %), although fulminant myocarditis caused by influenza infection is an uncommon type of myocarditis. We demonstrated a high prevalence of fulminant myocarditis

among all the myocarditis patients (17/29, 59 %). We found that, along with pneumonia and encephalopathy, myocarditis was an important cause of clinical deterioration in patients infected with H1N1pdm2009 in Japan [9, 10, 13, 15]. The influenza A virus might be more commonly associated with severe forms of myocarditis in the pandemic season than other seasons [2–4, 13, 15–17]. Because there was no significant difference in the H1N1pdm2009 virus in the 2009/2010 and 2010/2011 seasons [10, 12], we speculate that the pathological mechanism of influenza myocarditis differs depending on the pathogen, and may depend on host immunity, as indicated by anti-H1N1pdm2009 titers.

In our study, quantitative values of troponin I/T were elevated in all five patients in whom it was measured (100 %). Conversely, the qualitative quick troponin test was positive in only three of the six patients (50 %) in whom it was measured. Hence, we recommend that quantitative troponin I/T assays may be useful for the diagnosis and management of myocarditis.

Many kinds of viruses have been implicated as causes of myocarditis, with different viruses having different potentials to cause myocarditis [2–7, 12, 13, 15–17]. In their study, Bowles et al. reported that endomyocardial biopsy samples from 624 patients with clinically defined myocarditis were analyzed by PCR to detect various viral genes, of which 239 samples were positive [4]. Adenovirus was detected from 142 samples, enteroviruses from 85 samples, cytomegalovirus from 18 samples, and influenza A from 5 samples (0.8 %) [4]. In the present study, RT-PCR testing for H1N1pdm2009 from heart specimens were negative in both patients in which it was performed. Although it is well known that coxsackie viruses present a high affinity for cardiac myocytes, the pathological effects of influenza virus myocarditis in humans and mice are reportedly milder than coxsackie virus myocarditis and are more localized [4–6, 18]. The affinity of the influenza virus for cardiac myocytes is also reportedly low [3, 4, 6, 18]. Pan et al. reported the molecular mechanism of myocarditis associated with the influenza virus and revealed the importance of trypsin induction and increased production of pro-inflammatory cytokines in the pathogenesis of acute myocarditis [17–20]. Besides the direct effect of influenza virus infection, pro-inflammatory cytokines are thought to contribute to the pathogenesis of severe clinical features, including severe cardiac dysfunction, in influenza patients [13, 15, 18–21].

Myocarditis was proved by endomyocardial biopsy or autopsy in nine patients in this study, although the pathological findings were mild even in clinically defined fulminant myocarditis patients. A new approach to diagnosing myocarditis is cardiovascular magnetic resonance imaging (MRI) [1, 15]. MRI was indicative of myocarditis in one of two (50 %) patients in this study in whom it was performed. Hence, MRI might be more useful than invasive cardiac biopsy for diagnosing H1N1pdm2009 myocarditis and for estimating the activity and severity of inflammation, although further evaluation of its diagnostic efficacy is recommended.

There are some limitations to this study. We planned a statistical analysis between the number of myocarditis patients and the number of hospitalizations associated with H1N1pdm2009 influenza. However, this was not possible because of the low response rate to the question about the number of hospitalizations (responses were obtained from only 40 hospitals); further, many hospitalizations in the 2009/2010 season were for social reasons rather than serious illness.

Our study suggests that because cardiac symptoms developed within 3 days of sickness in 17 patients and cardiac dysfunction rapidly progressed in H1N1pdm2009 myocarditis, early diagnosis and prompt treatment of acute myocarditis with heart failure is required in patients with influenza infection during the pandemic season. Appropriate intervention in patients with fulminant influenza myocarditis consists of treatment with neuraminidase inhibitors to eliminate the causative virus, and mechanical circulatory support [intraaortic balloon pumping (IABP)/ percutaneous cardiopulmonary support (PCPS)] to treat the depressed myocardial function [1, 7, 12, 13, 15].

In conclusion, we confirmed the clinical features of patients with clinically defined myocarditis associated with influenza H1N1pdm2009 and demonstrated the high prevalence of fulminant disease (17/29, 59 %) in patients with influenza myocarditis. The number of patients with myocarditis associated with influenza A virus seemed to increase in the pandemic season but not in the nonpandemic season.

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Conflict of interest None declared.

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Review Article

Myocarditis Associated with Influenza A H1N1pdm2009

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Acute myocarditis is a well-known complication of influenza infection. The frequency of myocardial involvement in influenza infection varies widely, with the clinical severity ranging from asymptomatic to fulminant varieties. The worst cases can result in death due to impaired cardiac function, although such fulminant myocarditis associated with influenza infection is rare, as shown by previous papers. Following the 2009 influenza pandemic, we reported on the clinical features of a cohort of 15 patients in Japan with H1N1pdm2009 myocarditis. In our subsequent survey of the literature for case reports or series of patients with myocarditis associated with H1N1pdm2009, we identified 58 detailed cases. We discuss here the high prevalence of fulminant myocarditis (36/58, 62%) among patients reported to have myocarditis associated with H1N1pdm2009. Mechanical circulatory support was required in 17 of the patients with fulminant myocarditis, 13 of whom recovered. We stress the need for increased awareness of influenza-associated myocarditis; such knowledge will facilitate earlier diagnosis and treatment of this fatal complication during future influenza pandemics.

1. Introduction

Acute myocarditis is a well-known complication of influenza infection. The clinical expression varies from asymptomatic to fulminant myocarditis, which can result in severe hemodynamic dysfunction, necessitating high-dose catecholamines and mechanical circulatory support [1–11]. Pathogens frequently associated with myocarditis include coxsackievirus and adenovirus; fulminant myocarditis resulting from influenza A viral infection is rare, as shown by previous literature [1–23]. Our interest in influenza-associated myocarditis follows from our experience with the influenza pandemic of 2009 [3, 24–30]. We surveyed the literature for case reports and series involving myocarditis associated with H1N1pdm2009, and identified 58 patients with such a diagnosis [3, 31–62]. In the present study, we review the clinical, laboratory, and pathologic characteristics of these 58 patients and theorize about the pathogenesis of influenza myocarditis [63–68].

2. Cardiac Involvement of Influenza Infection before the 2009 Pandemic

Myocarditis was a common and sometimes fatal complication of influenza infection in the pandemics of the previous century [1–7]. Small autopsy-based studies on fatal cases revealed a complication rate of focal to diffuse myocarditis of 39.4% during the 1957 Asian influenza pandemic and 48% during the Spanish influenza pandemic [4–6]. All of these fatal cases with myocarditis also had severe pneumonia and multiple organ involvement. Thus, myocarditis is likely to be a terminal complication of pandemic influenza infection.

On the other hand, while many people are affected by seasonal influenza every year, complications in nonrespiratory tissues (e.g., encephalopathy, myocarditis, and myopathy) occur only occasionally [1–7]. The frequency of myocardial involvement in influenza infection varies (0–10%) depending on the diagnostic criteria, and fulminant myocarditis associated with seasonal influenza infection is rare, as shown in previous papers [1–4, 9, 12, 13, 15–23]. Indeed, only two (2/505, 0.4%) myocarditis cases were reported in 505 children admitted with laboratory-confirmed influenza during the 2003/2004 season in Canada [16].

Only rarely are influenza viral antigens or genetic material detected in the myocardium. There has been only one case report in which seasonal influenza A RNA was detected in a myocardial biopsy [15]. Miura et al. detected viral antigen in the myocardium using immunohistochemical staining on an autopsied heart [18]. Bowles et al. screened

endomyocardial biopsy samples from 624 patients with clinically defined myocarditis using PCR for various viral genes. Among 239 samples that tested positive for viral genes, adenovirus was detected in 142 samples, enterovirus in 85 samples, and influenza A in only five samples (0.8%) [12]. Caforio et al. screened endomyocardial biopsy samples from 120 patients with histologically proven myocarditis using PCR to detect various viral genes. Among 31 samples that tested positive for viral genes, none contained influenza A or B virus (0%) [13]. Thus, the myocardial toxigenicity of the seasonal influenza virus seems to be rather weak.

3. Myocarditis Associated with Influenza H1N1pdm2009 in Japan

The Ministry of Health, Labor and Welfare (MHLW) of Japan confirmed only 198 deaths among about 20.61 million patients infected with influenza A H1N1pdm2009 in the pandemic season in Japan. They also confirmed that 15 of these deaths resulted from myocarditis associated with this pandemic strain [28, 29]. We previously reported 15 H1N1pdm2009 myocarditis patients and demonstrated their clinical features by conducting a cross-sectional national survey with assistance from all members of the Japanese Circulation Society (JCS) in the 2009/2010 influenza season [31]. Myocarditis was diagnosed using the Guidelines for Diagnosis and Treatment of Myocarditis (JCS 2009) [8]. Seven (47%) of the 15 myocarditis patients had no baseline disease. Myocarditis was proved by endomyocardial biopsy in six patients. Histological findings in these six patients included myocarditis with degenerated myocytes, infiltration of lymphocytes (ranging from mild to moderate, but not severe), and interstitial edema. We demonstrated a high prevalence of fulminant myocarditis with fatal arrhythmias and/or varying degrees of cardiogenic shock among the majority (10/15, 67%) of patients with myocarditis. Mechanical circulatory support with intra-aortic balloon pumping (IABP) and/or percutaneous cardiopulmonary support (PCPS) was emergently required in 10 patients. Eight of these 10 patients were successfully rescued with mechanical circulatory support, while the remaining two patients died. We demonstrated that, along with pneumonia and encephalopathy, myocarditis was an important cause of clinical deterioration in patients infected with the pandemic H1N1pdm2009 virus in Japan.

4. Myocarditis Associated with Influenza H1N1pdm2009 in the World

We reviewed the data of 58 patients (28 males and 30 females; mean age 32 years) with myocarditis associated with H1N1pdm2009 worldwide [3, 31–62] and identified a high prevalence of fulminant myocarditis (36/58, 62%) among them. The characteristics of these 58 myocarditis patients are summarized in Table 1. The mean age (32 years) of myocarditis patients associated with H1N1pdm2009 influenza was lower than the age of patients with seasonal influenza in the present study, indicating an age shift to a younger population in myocarditis patients during the pandemic [27–30, 32]. We speculate that the pathological mechanism of influenza myocarditis differs depending on the pathogen, and may depend on host immunity, as indicated by anti-H1N1pdm2009 titers.

Forty-two percent of these myocarditis patients had no baseline disease, and 23% had preexisting lung disease. The number of female patients was larger than the number of male patients, although general acute myocarditis is more common in males [69, 70]. Further, although pregnancy is reported to be a risk factor for deterioration of pandemic influenza infection, only one of the women in this paper was pregnant [38]. The mean interval from influenza onset to cardiac involvement was 5.4 days. Cardiac symptoms developed on the first to third day of sickness in 51% of myocarditis patients. Thirteen (24%) of the 58 cases were complicated by pneumonia. Most of these patients exhibited electrocardiogram (ECG) abnormalities, such as ST elevation (34%) and inverted T waves (24%). Fatal arrhythmias, such as ventricular fibrillation, ventricular tachycardia, and complete AV block, were recorded on the first day of hospitalization in 22% of the cases. Echocardiography revealed diffuse or focal left ventricular wall motion abnormalities in 90% of the patients. Mean ejection fraction was 25 ± 11%. Mortality rate was 24% (14 deaths/58 patients). Coronary studies were performed in 41% of these patients (64% of adult patients), all of which were normal with the exception of one case with a chronic total lesion. Myocarditis was proved by endomyocardial biopsy and/or autopsy in 14 patients. Myocardial biopsy did not contribute to the diagnosis of myocarditis in several cases. In the six patients in whom endomyocardial biopsy was performed, the pathological findings were mild even in clinically defined fulminant myocarditis patients, compared with general myocarditis patients reported in previous papers [71, 72]. Although immunohistology has been acknowledged to have a substantially higher sensitivity, we did not have detailed information on the immunohistological analysis of biopsies [73, 74].

Cardiovascular magnetic resonance imaging (CMR) was used as the diagnostic tool in several cases with pericardial/myocardial involvement during H1N1pdm2009 infection [47–50, 55, 58, 73–75]. A neuraminidase inhibitor (either oseltamivir, zanamivir, or peramivir) was used in 85% of the cases. A left ventricular assist device (LVAD) or PCPS was used in 10 cases, and IABP was used in 11 cases. Extracorporeal lung assist with extracorporeal membrane oxygenation (ECMO) was used in 12 cases. Mechanical circulatory support (PCPS or LVAD and/or IABP) was used in 17 of the patients with fulminant myocarditis, 13 of whom were rescued. Patchy hemorrhage was demonstrated in three autopsy cases. Reverse transcriptase polymerase chain reaction (RT-PCR) for H1N1pdm2009 from heart specimens tested positive in four cases [44, 51, 53, 58].

In the 2009 pandemic, the rate of cardiac complications seemed to be higher than that reported for seasonal influenza A virus infection. Randolph et al. reported that acute myocarditis associated with H1N1pdm2009 (1.4% of 838 cases) was an independent risk factor for death in children (<21 years old) admitted to a PICU in the USA [61].

Table 1: Detailed characteristics of 58 patients with myocarditis associated with H1N1pdm2009 influenza.

Characteristics of 58 patients with H1N1pdm2009 influenza reported in detail	Result (%)
Age (mean, years) (range)	32 (3–72)
Less than 17 years (%)	14 cases (24%)
Sex (% female)	30 cases (52%)
Death (%)	14 cases (24%)
Interval between influenza onset and cardiac symptoms (mean, days) (range)	5.4 (1–21)
1st day to the 3rd day (%)	51%
Cardiac symptoms	
Dyspnea (%)	54%
Chest pain (%)	30%
Fulminant myocarditis (%)	36 cases (62%)
Mortality rate of patients with fulminant myocarditis	39% (14/36)
Pneumonia as a complication (%)	13 cases (22%)
ECG findings on the first day of hospitalization	
ST elevation (%)	34%
T inversion (%)	24%
Fatal arrhythmias (VF, VT, complete AV block) (%)	22%
Echocardiogram	
Diffuse or focal left ventricular wall motion abnormalities	90%
Ejection Fraction (mean ± SD)	25 ± 11%
Percentage of patients in whom CAD was ruled out by CAG	41%
Percentage of adult patients in whom CAD was ruled out by CAG	64%
Treatment	
Neuraminidase inhibitors	85%
PCPS	10 cases (17%)
LVAD	1 case (1.7%)
IABP	11 cases (19%)
PCPS or LVAD and/or PCPS	17 cases (29%)
Mortality of patients treated with mechanical support	23% (4/17)
ECMO	12 cases (21%)
Biopsy	10 cases (17%)
Myocarditis with lymphocyte infiltration (mild~moderate)	6 cases
No myocarditis (according to the Dallas criteria)	4 cases
Autopsy	8 cases (14%)
Pachy hemorrhage in the autopsied heart	3/8 cases (38%)
RT-PCR positivity rate for H1N1pdm2009 virus from heart specimens	4 cases

ECG: electrocardiogram; VF: ventricular fibrillation; VT: ventricular tachycardia; AV block: atrioventricular block; CAD: coronary artery disease; CAG: coronary angiography; PCPS: percutaneous cardiopulmonary support; LVAD: left ventricular assist device; IABP: intra-aortic balloon pumping; ECMO: extracorporeal membrane oxygenation; RT-PCR: reverse transcription polymerase chain reaction.

Bratincsák et al. reported four patients with myocarditis associated with H1N1pdm2009 within a 30-day period in 2010 and suggested that H1N1pdm2009 virus might be more commonly associated with myocarditis than seasonal influenza virus [58]. Zheng et al. reported finding seven children (5%) with complicated myocarditis among 148 children hospitalized with influenza H1N1pdm2009 infection in China [62]. Shin et al. analyzed a group of 30 critically ill pediatric patients in Korea and reported that the most common causes of death were encephalopathy (four children) and myocarditis (four children) [63]. Martin et al. examined a cohort of 123 hospitalized patients infected with H1N1pdm2009 and reported that six patients (4.9%) had either new or worsened left ventricular dysfunction. They concluded that reversible cardiac dysfunction is a relatively common complication associated with H1N1pdm2009 [60]. Thus, the frequency of cardiac involvement in influenza virus infection is likely elevated with influenza H1N1pdm2009 compared to seasonal influenza.

5. Theories of Pathogenesis of Influenza Myocarditis

It is well known that coxsackieviruses present a high affinity for cardiac myocytes [9, 12–14]. There is a distinct difference in the pathological findings between myocarditis associated

with influenza A virus and myocarditis associated with coxsackieviruses [1–14]. The pathological effects of influenza viral myocarditis in humans and mice are reportedly milder and are more localized than those seen in coxsackievirus myocarditis [8, 9, 14]. Kotaka et al. reported that murine influenza myocarditis was histologically mild and brief in duration compared to coxsackievirus B3 myocarditis [64]. Electron microscopic findings of the heart from a murine influenza myocarditis model showed many infiltrating lymphocytes directly attached to the cardiac myocytes. Nonetheless, the affinity of the influenza virus for cardiac myocytes appears to be low.

Pan et al. investigated the molecular mechanism of myocarditis associated with the influenza virus and revealed the importance of trypsin induction and increased production of matrix metalloproteinase (MMP) and proinflammatory cytokines in the pathogenesis of acute myocarditis [65–68]. Pan et al. also showed that inhibition of trypsin suppressed viral replication, upregulated of MMPs and cytokines, and significantly improved the cardiac function of mice infected with influenza A virus [65–68]. Teijaro et al. revealed immune cell infiltration and cytokine production as distinct events, both of which are orchestrated by endothelial cells [68]. Beside the direct effect of influenza virus infection, proinflammatory cytokines and endothelial cell dysfunction are thought to contribute to the pathogenesis of severe clinical features, including severe cardiac dysfunction and encephalopathy in patients infected with influenza virus [65–68].

Calore et al. observed perivascular hemorrhage of the brain in five of six autopsies of H1N1pdm2009 cases; focal myocarditis was also observed in one case [45]. They suggested that hemorrhagic lesions in the brain might be due to vascular lesions or to an increase in endothelial permeability. Edler et al. demonstrated that an autopsy of a fulminant myocarditis case showed small patch-shaped hemorrhages on the top of the heart and a florid myocarditis with marked mixed-cell infiltrates; H1N1pdm2009 virus was detected in the brain and heart by RT-PCR [53]. RT-PCR from the myocardium showed positive results in four of the patients surveyed in the present paper. Thus, although the pathogenesis of influenza-associated myocarditis remains unclear, the literature suggests that endothelial dysfunction may be important in the pathogenesis of myocarditis and encephalopathy associated with influenza virus.

6. Diagnosis of Myocarditis Associated with Influenza A Virus

In the present paper, chest pain or worsening dyspnea was a common symptom in patients with myocarditis associated with H1N1pdm2009. Cardiac symptoms (e.g., dyspnea, cough, palpitation, and impaired consciousness) developed from the first sick day to the third sick day in 51% of patients. On the other hand, cardiac dysfunction reportedly developed after recovery from flu-like symptoms in two patients. Since cardiac dysfunction progressed rapidly in H1N1pdm2009 myocarditis, early diagnosis and prompt treatment of acute myocarditis with heart failure are required in patients with influenza infection during the pandemic season.

The ECG is a sensitive and convenient tool for diagnosis of myocarditis. ST elevation, T inversion, and conduction block are frequently observed. The ECG and echocardiogram must be repeated for the diagnosis of myocarditis in patients with suspected myocarditis; ECG monitoring is also useful to detect fatal arrhythmias [7, 8]. Myocarditis can be confirmed by observation of transient wall thickening, reduced wall motion, and reduced cardiac chamber size in addition to pericardial effusion on echocardiography [8, 76]. Erden et al. reported that tissue Doppler echocardiography is useful to detect subclinical cardiac dysfunction [77]. It is also important to perform echocardiography to distinguish fulminant myocarditis, which is a lethal disease, from acute myocarditis. Felker et al. reported that patients with fulminant myocarditis had near normal diastolic dimensions with increased septal thickness, while those with acute myocarditis had increased diastolic dimensions with normal septal thickness [11].

Myocarditis is confirmed by the findings of transient elevation of creatinine kinase (CPK), the MB form of creatinine kinase (CPK-MB), and cardiac troponin. Brown et al. reported the usefulness of troponin as a diagnostic test in patients in the pediatric emergency department who report chest pain, although troponin is not useful for excluding cardiac ischemic disease in adults [78]. Erden et al. and Sahin et al. reported acute myocarditis mimicking acute myocardial infarction associated with H1N1pdm2009 infection, with chest pain and ST elevation, suggesting that coronary artery disease should be excluded in cases with severe chest pain [35, 42]. Coronary artery disease was excluded by coronary study in 64% of the adults in this paper. For even more definitive diagnosis, endomyocardial biopsy should be performed after the coronary lesion has been excluded, although it is not essential for the clinical diagnosis of myocarditis. However, even if the results of cardiac biopsy are negative, the presence of myocarditis cannot be excluded due to the possibility of sampling error. Baccouche et al. reported that CMR and endomyocardial biopsy have good diagnostic performance as single techniques in patients with Troponin-I-positive acute chest pain in the absence of coronary artery disease [74]. Liu and Yan observed that CMR, a new technique, is helpful for the detection of myocarditis, because CMR can visualize the entire myocardium [75]. Gutbertet et al. reported that although CMR imaging may be helpful in noninvasively detecting intramyocardial inflammation, it fails to detect viral persistence [73]. Takeuchi et al. reported that MRI might be more useful than invasive cardiac biopsy for diagnosing H1N1pdm2009 myocarditis and for estimating the activity and severity of inflammation [49]. Mavrogeni and Manoussakis recommended CMR for its sensitivity in detecting pericardial/myocardial involvement during H1N1pdm2009 infection, especially if echocardiographic evaluation is negative [55]. Thus, CMR may be useful for the diagnosis of influenza virus myocarditis, because the affinity of influenza virus for cardiac myocytes appears to be low, identifying the intramyocardial inflammation of influenza myocarditis.

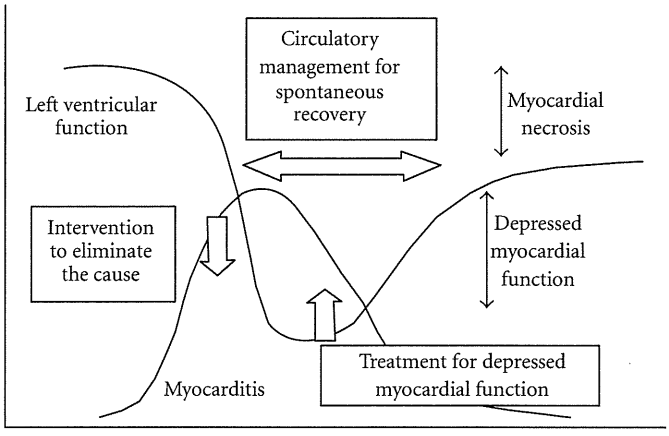

FIGURE 1: Course of cardiac dysfunction and timing of intervention in myocarditis (Guidelines for Diagnosis and Treatment of Myocarditis (JCS2009)).

Usually viral infection is diagnosed if the viral antibody titer is at least four times higher in an acute phase serum sample than that in a sample obtained in the remission phase (with samples collected at least two weeks apart). Although diagnosis of the pathogen in myocarditis is difficult, good diagnostic methods, including a rapid diagnostic test for influenza virus and an RT-PCR assay for influenza H1N1pdm2009 virus from a nasal swab, were already available during the 2009 pandemic. In fact, the prevalence of these new diagnostic methods may be one of the reasons why the number of case reports of myocarditis increased in this pandemic. RT-PCR of the myocardium is more useful for identifying the genomes of viruses causing myocarditis than other methods and showed positive results in 4 patients in our paper [44, 51, 53, 58].

7. Treatment of Myocarditis Associated with Influenza

The course of cardiac dysfunction and timing of intervention are described in the Guidelines for Diagnosis and Treatment of Myocarditis of the Japan Circulation Society (JCS2009) and are shown in Figure 1. Myocarditis is treated in three ways: (1) intervention to eliminate the cause, (2) intervention to improve hemodynamic compromise, and (3) intervention for cardiac dysfunction [8]. To eliminate the cause, forty-one (85%) of the myocarditis patients in our survey were treated with neuraminidase inhibitors. Treatment with neuraminidase inhibitors is also recommended by the Japanese Association of Infection for all patients infected with influenza [29]. The low-case fatality rate in Japan could be a result of aggressive early intervention with antiviral drugs, such as oseltamivir and zanamivir [28, 29]. Morioka et al. reported no cases of influenza-associated encephalopathy or myocarditis in 44 infants aged <3 months treated with oseltamivir for H1N1pdm2009 infection in Japan [30] and therefore recommended oseltamivir as safe and efficacious for use in infants <3 months of age. Use of immunosuppressive therapy is controversial for both acute myocarditis and influenza infection [8, 24, 27–30, 79, 80]. High-dose steroids are not recommended, because of unproven benefit and potentially harmful effect on influenza infection.

The first therapy for myocarditis patients with heart failure is supportive intervention in potentially fatal cases. The recent application of PCPS and/or IABP in serious cases of viral myocarditis has yielded good outcomes. LVAD or PCPS was used in 10 cases, and IABP was used in 11 cases in this paper. Extracorporeal lung assist (ECMO) was used in 12 cases. These mechanical circulatory devices can be used to decrease mortality and as a bridge to transplantation. Seventeen H1N1pdm2009 myocarditis patients were treated with mechanical circulatory support, thirteen (76%) of whom survived. On the other hand, in the national survey of fulminant myocarditis in Japan, 30 of 52 patients (57.7%) survived without antiviral treatment [10]. Because of the nature of the study design, it was difficult to show that the neuraminidase inhibitors significantly improved the survival rate of patients with fulminant myocarditis associated with influenza. Based on our survey, we recommend that patients with fulminant myocarditis be treated with a combination of neuraminidase inhibitors (to eliminate the causative virus) and mechanical circulatory support (to treat depressed myocardial function).

8. Conclusion

We reviewed the details of 58 cases of myocarditis associated with H1N1pdm2009 and found a high prevalence of fulminant myocarditis (36/58, 62%) among them; 14 of these 58 patients died. Diagnosis and treatment during this pandemic were facilitated by improved diagnostic methods (e.g., rapid diagnostic tests, RT-PCR for influenza virus, echocardiogram, and CMR) and by the ready availability of treatment with neuraminidase inhibitors and mechanical circulatory support. We stress the need for increased awareness of influenza-associated myocarditis. Such knowledge will facilitate earlier diagnosis and treatment of this potentially fatal complication during future influenza pandemics.

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Original Article

Increased symptom severity but unchanged neuraminidase inhibitor effectiveness for A(H1N1)pdm09 in the 2010–2011 season: comparison with the previous season and with seasonal A(H3N2) and B

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Background No studies of the clinical symptoms before starting therapy or of the effectiveness of neuraminidase inhibitors (NAIs) have been carried out of the 2009–2010 and 2010–2011 seasons that compare A(H1N1)pdm09 or the three circulating types of influenza virus.

Methods The clinical symptoms and duration of fever (body temperature ≥37·5°C) after the first dose of an NAI (oseltamivir, zanamivir, laninamivir) were analyzed. PCR was carried out for 365 patients with A(H1N1)pdm09 in the 2009–2010 season and for 388 patients with one of the three types of influenza circulating in the 2010–2011 season. IC_{50} for the three NAIs was also analyzed in 51 patients in the 2010–2011 season.

Results The peak body temperature was significantly higher in 2010–2011 than in 2009–2010 for patients under 20 years with A(H1N1)pdm09, and in the 2010–2011 season for children 15 years or younger with A(H1N1)pdm09 than for those with other virus types. The percentage of A(H1N1)pdm09 patients with loss of appetite or fatigue was significantly higher in 2010–2011 than in the previous season. The duration of fever was not affected by the kind of NAI or by age in multiple regression analysis. The percentage of patients afebrile at 48 hours after the first dose of NAI was significantly higher for A(H1N1)pdm09 than for A(H3N2) (laninamivir) or B (oseltamivir and laninamivir).

Conclusion Although the clinical symptoms of A(H1N1)pdm09 were slightly more severe in the 2010–2011 season, the effectiveness of the NAIs remained high in comparison with 2009–2010 and with other types of seasonal influenza.

Keywords A(H1N1)pdm09, clinical symptom, IC_{50}, laninamivir, neuraminidase inhibitor.

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Introduction

Influenza A(H1N1)pdm09 was highly prevalent in the 2009–2010 season, with few cases of A(H3N2) or B reported.[1,2] However, all three subtypes (types) spread widely and almost simultaneously in the 2010–2011 winter season.[1,3,4] Little study has been carried out of the differences in the clinical symptoms or the effectiveness of neuraminidase inhibitors (NAIs) between these two seasons for A(H1N1)pdm09 viruses or among the three influenza subtypes. A(H1N1)pdm09 was reported mainly in the autumn (mostly September–December) of the 2009–2010 season, but prevailed in winter (mostly January–March) in the 2010–2011 season, which is similar to the usual influenza season in Japan.[1] Therefore, we thought it would be interesting to determine how the clinical features of A(H1N1)pdm09 might have differed between these two seasons.

We have reported the usefulness of neuraminidase inhibitors (NAIs) almost annually[5–11] and have shown reduced effectiveness of oseltamivir in the 2008–2009 season, when the oseltamivir-resistant (H275Y NA mutation) A(H1N1) viruses were highly prevalent, compared with the previous