The post-infection outcomes of influenza and acute respiratory infection in patients above 50 years of age in Japan: an observational study

Hideyuki Ikematsu,[a,b] Yuriko Takeuchi,[c] Mats Rosenlund,[c] Naoki Kawai,[b] Ryuji Shimamura,[b] Miki Hirata,[b] Norio Iwaki[b]

[a]Department of Clinical Trials, Center for Advanced Medical Innovation, Kyushu University, Fukuoka, Japan. [b]Japan Physicians Association, Tokyo, Japan. [c]GlaxoSmithKline Biologicals, Wavre, Belgium.
Correspondence: Hideyuki Ikematsu, Department of Clinical Trials, Center for Advanced Medical Innovation, Kyushu University, 3-1-1, Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. E-mail: hikematsu@camiku.kyushu-u.ac.jp

Accepted 28 August 2011. Published Online 10 October 2011.

Objectives Influenza can be a serious illness, especially for older people, and reducing the impact of influenza in elderly is important. The objective of this study was to estimate the prevalence and postinfection outcomes of influenza among the over-50 population in Japan.

Design An observational study was designed to ascertain the proportion of influenza cases in a population aged ≥50 years with acute respiratory infection (ARI) and to determine the postinfection outcomes of their illness during the 2008–09 influenza season in Japan. Respiratory specimens obtained from a total of 401 patients were tested by PCR for influenza viruses, respiratory syncytial virus (RSV) and human metapneumovirus (hMPV). The effectiveness of the seasonal trivalent influenza vaccine was estimated by a test-negative case control analysis.

Setting Seventeen outpatient clinics located in four separate areas of Japan.

Sample Respiratory swab specimens from the ARI patients aged ≥50 years.

Main outcome measures Laboratory confirmed influenza in patients presenting with ARI.

Results In all, 89 (22.2%) of the patients were positive for one of the tested viruses; 70 (78.7%) with influenza, 17 (19.1%) with RSV, and 2 (2.2%) with hMPV. Cough (95.7% vs 73.4%), loss of appetite (67.1% vs 35.5%), absence from work (50.0% vs 23.0%), impact on daily activity (90.0% vs 62.5%), and caregiver absence from work (5.7% vs 0.6%) were observed higher in influenza patients. The duration of feeling weakness (6.3 ± 5.4 vs 3.6 ± 1.9 days) and average days of reduced activity (5.2 vs 3.6 days) were longer for influenza patients. Vaccine effectiveness was estimated to be 32.1% (95% CI: −14.9, 59.9%).

Conclusions Influenza was the dominant ARI-causing virus and the clinical and socio-economic outcomes imposed on patients over 50 years of age was high for influenza.

Keywords Acute respiratory infection, human metapneumovirus, influenza, influenza-like illness, post-infection outcomes, respiratory syncytial virus, test-negative case–control, vaccine effectiveness.

Introduction

Seasonal influenza is an important cause of morbidity and mortality.[1] Influenza viruses have the potential to cause not only epidemics but also occasional pandemics. While influenza viruses remain of high scientific interest, studies based on clinical features without laboratory confirmation of diagnoses may lack reliability because of the similarity and overlap of influenza symptoms with those of other etiological agents that can give rise to acute respiratory infections (ARIs).

Older people are at greater risk from complications arising from influenza than younger adults.[2] In the United States, the elderly account for a significant number of influenza-associated hospitalizations.[3] In Japan, a study of excess mortality associated with influenza epidemics across all ages during the period 1987–2005 estimated that 85–90% occurred among persons aged 65 and above.[4] With declining birth and death rates, Japan already has one of the highest rates of aging globally,[5] and it is projected that by 2050 over 30% of the Japanese population aged 60 or older will be 80 or above.[6] Recently, treatment

for influenza with neuraminidase inhibitors has become popular in Japan, and they are prescribed very often even for mild cases of influenza. However, there have been no studies addressing the post-infection outcomes of laboratory-confirmed seasonal influenza on those aged 50 and above under such special circumstance. We therefore conducted an observational study using a population of patients with ARI in this age group from a primary care setting. The primary objective was to determine the proportion of ARI cases that were affected by influenza among the study population during the 2008–2009 influenza season. Secondary objectives were to ascertain both the clinical and socioeconomic post-infection outcomes imposed on the subjects during their influenza episode. We also carried out a test-negative case–control analysis to assess the effectiveness of the trivalent influenza vaccine (TIV) in preventing influenza in this age group.

Methods

The observational study was conducted in compliance with Good Clinical Practice (GCP) guidelines and the Japanese Ministry of Health, Labor and Welfare's (MHLW) ethical guidelines for epidemiological research. A central Institutional Review Board (IRB) provided ethics review and approval. Seventeen outpatient clinics located in four separate areas of Japan, Fukuoka, Ishikawa, Gifu and Tokushima prefectures participated in the study. The recruitment period was from November 2008 to May 2009. Patients aged 50 years or over presenting with ARI were enrolled following informed consent. The inclusion criteria were a temperature of 37·5°C or more and/or feverishness at least one of the following respiratory symptoms: coryza and/or nasal congestion, cough, and sore throat (criteria adapted from the ARI subsection of 'influenza case definitions' specified by the European Centre for Disease Prevention and Control[7]).

Medical histories were collected to obtain baseline data on demographics, underlying medical conditions (pneumonia, chronic obstructive lung disease, asthma, immunocompromised, diabetes, dialysis, arteriosclerosis, coronary artery disease, cardiac failure, cerebrovascular disease, regular smoking), and whether the patient had received a TIV vaccination prior to the 2008–2009 influenza season. Clinical symptoms and medical histories were recorded by the physician in clinical interview and through the subjects' medical record. Participants were requested to keep a daily record of body temperature, clinical symptoms, medication taken and information on the socioeconomic outcomes of their ARI episode, such as reduced activity and workdays lost by themselves or their care givers. Follow-up contact was made by telephone 12–21 days after the initial visit, when participants were asked to complete a questionnaire

while referring to their diary as a memory aid. Interviewers were blinded to the laboratory test results of the subjects. All subjects participating in the study were accounted for and followed up.

Two respiratory swab specimens were taken, one for rapid testing as part of the clinical diagnosis and the other for subsequent laboratory testing. Specimens for laboratory testing were collected and sent to the central laboratory at Hara-doi Hospital by designated courier. All clinical specimens were labeled, handled, analyzed, and stored in accordance with GCP and JNIPH guidelines and standard operating procedures (SOPs). Agreement between rapid diagnosis kit and laboratory confirmation was analyzed to assess any misclassification of disease.

Clinical samples were tested for influenza, respiratory syncytial virus (RSV), and human metapneumovirus (hMPV) by reverse transcription polymerase chain reaction (RT-PCR). The PCR mixture comprised 7·5 μl nuclease-free water, 6·2 μl GoTaq® Green Master Mix, 2X (Promega KK, Promega Corp., Madison, WI, USA), 0·15 μl forward primer, 0·15 μl reverse primer, and 1·0 μl template. Detection and subtyping of influenza type A (H1N1 and H3N2) and type B viruses were carried out as described by Stockton *et al.*[8] Amplification conditions consisted of initial denaturation at 94°C for 10 seconds followed by 94°C for 5 seconds, 53°C for 20 seconds, and 72°C for 20 seconds applied for 32 cycles in the first round and 28 cycles in the second round, and final extension at 72°C for 10 seconds.

Infections by RSV were detected as described by Falsey *et al.*[9] Amplification conditions consisted of initial denaturation at 95°C for 10 seconds followed by 95°C for 5 seconds, 42°C for 20 seconds, and 72°C for 20 seconds applied for 32 cycles in the first round and 28 cycles in the second round, and final extension at 72°C for 10 seconds. Infections by hMPV were detected as described by Peret *et al.*[10] Amplification conditions consisted of initial denaturation at 94°C for 10 seconds followed by 94°C for 5 seconds, 55°C for 20 seconds, and 72°C for 20 seconds applied for 40 cycles in the first round and 40 cycles in the second round, and final extension at 72°C for 10 seconds.

The statistical software used were sas 8.2 (or later versions) and STATA/SE 10. Standard parametric techniques employed for statistical analysis (including comparison of baseline characteristics) were χ^2, Fisher's exact and Student's *t*-tests. With the power of the study set at 80% and significance level at 5%, the minimum sample size was estimated to be 500.

To evaluate crude estimates of the effectiveness of the seasonal vaccine in preventing influenza, we conducted a test-negative case–control analysis using the data obtained for the study population of patients with ARI who were infected with one of the three influenza viruses contained in the seasonal TIV (A/H1N1, A/H3N2, and B). The

controls were patients with ARI who tested influenza negative. Participants were considered vaccinated if they had received the TIV at least 14 days before presenting with ARI.

Results

A total of 401 patients were enrolled into the study, 233 (58·1%) women and 168 (41·9%) men. A comparison of detailed baseline characteristics of the study population grouped by influenza diagnosis revealed that baseline characteristics of the influenza and non-influenza groups were similar in terms of age, gender, and vaccination status (Table 1). The two groups were also similar with respect to the presence of an underlying medical condition (45·7% and 43·2%, respectively). The medical conditions did not differ significantly between the two groups (data not shown).

In all, 89 (22·2%) of the 401 study participants were found to be infected with one of the tested viruses: 70 (78·7%) with influenza, 17 (19·1%) with RSV, and 2 (2·2%) with hMPV. According to the assessment of possible misclassification of disease between the rapid diagnosis kit and laboratory confirmation, specificity, sensitivity, positive predictive value, and negative predictive value were 93·0%, 78·6%, 70·5%, and 95·3%, respectively. Only laboratory-confirmed influenza was categorized as influenza positive for further analyses. Among the 70 patients positive for influenza, H1N1, H3N2, and B were detected in 33

(47·1%), 33 (47·1%), and 4 (5·7%) cases, respectively. Influenza was diagnosed in 70 (17·5%) of the 401 participants, of whom 28 (40·0%) had received the seasonal vaccination, while 42 (60·0%) had not. Among the 70 patients positive for influenza, neuraminidase inhibitors were prescribed at the initial visit to 50 (71·4%), of whom 23 (46·0%) had been vaccinated, but 27 (54·0%) had not. Among the 331 influenza-negative patients, 164 (49·5%) had been vaccinated and 167 (50·5%) had not (Table 1).

Significant differences were observed between influenza and non-influenza cases with regard to both clinical symptoms and socioeconomic post-infection outcomes (Table 2). The prevalence of cough, headache, loss of appetite, and both the feeling and duration of weakness were significantly higher among influenza-positive patients: Cough was observed in 95·7% and 73·4% ($P < 0·01$), headache in 64·3% and 49·5% ($P = 0·03$), loss of appetite in 67·1% and 35·5% ($P < 0·01$), feeling of weakness in 32·9% and 20·5% ($P = 0·03$), of influenza positive and negative, respectively. The duration of feeling weakness was 6·3 ± 5·4 days for positive and 3·6 ± 1·9 days ($P < 0·01$) for negative participants. Although there was no significant difference, it is particularly worth noting that the total duration of illness was more than 2 weeks in influenza-positive patients probably due to the vulnerability of the study population of this age. In terms of the socioeconomic outcomes of their ARI episode, absence from work, impact on daily activity, and caregiver absence from work were all reported to be significantly higher for those with influenza: Absence from work were observed in 50·0% and 23·0% ($P < 0·01$), impact on daily activity in 90·0% and 62·5% ($P < 0·01$), caregiver absence from work in 5·7% and 0·6% ($P = 0·010$) of influenza positive and negative, respectively (Table 2). Average days of absence was 3·1 days and 2·2 days ($P = 0·026$), and days of reduced activity was 5·2 days and 3·6 days ($P < 0·001$) for influenza-positive and influenza-negative participants. When the clinical symptoms and socioeconomic outcomes of only the influenza-positive cases were compared between those vaccinated or unvaccinated in the 2008–2009 season, there were no significant differences for any of the clinical or socioeconomic parameters (Table 3).

The test-negative case–control analysis of vaccine effectiveness indicated that the 2008–2009 seasonal influenza vaccination was 32·1% (−14·9, 59·9%) effective in preventing influenza in the study population overall. We were unable to determine age-specific vaccine effectiveness because of the small number of influenza cases in each age group.

Discussion

This is the first study in Japan to quantify the post-infection outcomes of seasonal influenza confirmed by labora-

Table 1. Characteristics of Japanese patients with ARI in 2008–2009 by influenza infection status

Characteristics	Influenza (+)* (n = 70)	Influenza (−)** (n = 331)	Difference (P-value)
Age			
Average	63·1	65·0	0·330
Range	50–84	50–95	
50–64 years	43 (61·4%)	171 (51·7%)	
65–74 years	18 (25·7%)	107 (32·3%)	
≥75 years	9 (12·9%)	53 (16·0%)	
Gender			
Males	29 (41·4%)	139 (42·0%)	0·931
Females	41 (58·6%)	192 (58·0%)	
Underlying medical condition			
Yes	32 (45·7%)	143 (43·2%)	0·700
No	38 (54·3%)	188 (56·8%)	
Vaccinated for influenza 2008–2009 season			
Vaccinated	28 (40·0%)	164 (49·5%)	0·146
Unvaccinated	42 (60·0%)	167 (50·5%)	

ARI, acute respiratory infection.
*Influenza positive.
**Influenza negative.

Table 2. Clinical symptoms and socioeconomic outcomes in 401 patients with ARI in Japan 2008–2009 by laboratory confirmation

	Influenza (+) (*n* = 70)*	Influenza (−) (*n* = 331)**	OR (95%CI)	*P*-value
Clinical symptoms				
Coryza and/or nasal congestion	53 (75·7%)	235 (71·0%)	1·27 (0·70–2·31)	0·43
Cough	67 (95·7%)	243 (73·4%)	8·09 (2·48–26·37)	<0·01
Headache	45 (64·3%)	164 (49·5%)	1·83 (1·07–3·13)	0·03
Loss of appetite	47 (67·1%)	117 (35·5%)	3·74 (2·16–6·46)	<0·01
Myalgia	42 (60·0%)	160 (48·3%)	1·60 (0·95–2·71)	0·08
Sore throat	50 (32·9%)	256 (77·3%)	0·73 (0·41–1·31)	0·29
Feeling of weakness	23 (32·9%)	68 (20·5%)	1·89 (1·08–3·33)	0·03
Duration of feeling of weakness (days)	6·3 ± 5·4	3·6 ± 1·9	2·09 (1·14–3·83)	<0·01
Total duration of illness (days)	19·0 ± 3·4	18·4 ± 5·7	1·41 (0·64–3·12)	0·39
Socioeconomic outcomes				
Absence from work	35 (50·0%)	76 (23·0%)	3·36 (1·97–5·72)	<0·01
Days absent (average)	3·1	2·2	1·62 (1·04–2·54)	0·026
Impact on daily activity	63 (90·0%)	207 (62·5%)	5·93 (2·39–12·14)	<0·01
Days of reduced activity	4	3	–	–
Days of reduced activity	5·2	3·6	1·51 (1·18–1·93)	0·001
Caregiver absence from work	4 (5·7%)	2 (0·6%)	9·97 (1·79–55·55)	0·010
Days absent (average)	3·5	2	3·15 (0·16–63·12)	0·492

ARI, acute respiratory infection.
*Influenza positive.
**Influenza negative.

tory testing of clinical respiratory specimens in a cohort of patients with ARI aged 50 and above. Because the symptoms of influenza are similar to those arising from other viral respiratory pathogens, diagnostic respiratory samples obtained from each participant were laboratory-confirmed using RT-PCR, which is highly sensitive and specific for detecting influenza viruses, RSV, and hMPV.[8,9] The latter two commonly cocirculate with influenza in winter months and give rise to similar symptoms, which can be severe in the elderly.[2,9]

The results showed that in the study population of 401 patients with ARI, 70 (17·5%) were influenza positive. Among the respiratory viruses tested for and identified, influenza was dominant (almost 80·0%), which agrees with previous reports relating to the elderly.[2,11] In our study, influenza viruses were four times more prevalent than RSV and 35 times more common than hMPV. The features may vary by studied season or year. Because the study was conducted in the flu season, a possible over-representation of influenza prevalence cannot be eliminated. There is a lack of reliable comparative data in Japan on the prevalence of laboratory-confirmed influenza among ARI cases, but studies conducted elsewhere[12,13] support our finding that influenza is the predominant ARI-causing viral pathogen among the viruses tested for.

Our study demonstrated that influenza-positive patients suffered more severe outcomes in terms of clinical symp-

toms than patients with ARI who were influenza negative including those who may have been infected with other viruses and those with no pathogen detected. In particular, the prevalence of cough, headache, loss of appetite, feeling of weakness, and duration of weakness was all significantly greater for those with laboratory-confirmed influenza, even though anti-influenza drug was prescribed to 50 of the 70 influenza-positive cases (71·4%). Previous studies have also reported that elderly influenza patients suffered from longer-lasting coughs[14] and that weakness was a common symptom of influenza.[15] The socioeconomic outcomes on influenza-positive patients was also significantly greater than for the non-influenza patients with ARI in terms of impact on daily activity and the absence from work by both the patient and caregiver. It is particularly noteworthy that half of the influenza patients reported being absent from work (with a median duration of 3 days), whereas less than a quarter of the non-influenza ARI patients reported work days lost (median duration 2 days). In Japan, the majority of elderly people continue to do some form of work. Sometimes full-time worker, but part-time jobs and volunteering are regarded as work. The number of days of absenteeism of other persons who provided patient care during the follow-up period was analyzed because it is quite common for people around patients to care for the patient because of the limited availability of home nursing service in Japan. Patient absenteeism and the need for care-

Table 3. Clinical symptoms and socioeconomic outcomes of influenza-positive vaccinated and unvaccinated patients in Japan 2008–2009

	Vaccinated (n = 28)	Unvaccinated (n = 42)	OR (95% CI)	P-value
Clinical symptoms				
Coryza and/or nasal congestion	23 (82·1%)	30 (71·4%)	1·84 (0·57–5·96)	0·31
Cough	26 (92·9%)	41 (97·6%)	0·32 (0·03–3·68)	0·56
Headache	17 (60·7%)	28 (66·7%)	0·77 (0·29–2·09)	0·61
Loss of appetite	19 (67·9%)	28 (66·7%)	1·06 (0·38–2·93)	0·92
Myalgia	15 (53·6%)	27 (64·3%)	0·64 (0·24–1·70)	0·37
Sore throat	18 (64·3%)	32 (76·2%)	0·56 (0·20–1·61)	0·28
Feeling of weakness	10 (35·7%)	13 (31·0%)	1·24 (0·45–3·41)	0·68
Duration of feeling of weakness (days)	8·1 ± 6·7	5·2 ± 4·4	1·62 (0·69–3·82)	0·27
Total duration of illness (days)	18·6 ± 3·2	19·3 ± 3·6	0·32 (0·02–4·60)	0·41
Socioeconomic outcomes				
Absence from work	14 (50·0%)	21 (50·0%)	1·00 (0·38–2·60)	1·000
Days absent (average)	3·3	2·9	1·49 (0·45–4·94)	0·52
Impact on daily activity	24 (85·7%)	39 (92·9%)	0·46 (0·09–2·24)	0·43
Days of reduced activity (average)	5·5	4·9	1·15 (0·69–1·94)	0·59
Caregiver absence from work	2 (7·1%)	2 (4·8%)	1·54 (0·20–11·61)	1·00
Days absent (average)	1·5	5·5	–	0·13

givers to also take time off work appears to have also been substantially higher in the influenza-positive group. The burden of reduced daily activity would be reflected in diminished performance – with its consequent economic impact and loss of productivity, as well as intruding on the quality of life of both patients and their care givers.

Comparison of the clinical symptoms and socioeconomic criteria between vaccinated and unvaccinated influenza-positive participants revealed no significant differences. In addition, the proportion of vaccinated and unvaccinated patients was similar among those who had antiviral prescriptions (23/50 versus 27/50, respectively). Among this group of above 50 years of age, the seasonal vaccine appeared to have only a limited mitigating effect regarding clinical symptoms and reducing the socioeconomic outcomes of influenza.

To assess the effectiveness of the 2008–2009 TIV seasonal vaccine, we employed a test-negative case–control analysis in which the control group consisted of all the ARI participants who tested negative for influenza. Such a design is relatively easy to implement, controls better for bias related to healthcare service utilization than the traditional case–control method and has been shown to provide accurate estimates of vaccine effectiveness.[16] The findings on the effectiveness of TIV in preventing influenza in this age group were inconclusive [32·1% (−14·9, 59·9%)]. However, this study was not primarily designed to study vaccine effectiveness, and these results should be interpreted with caution given the limited power and potential bias including lack of control for confounding. Nevertheless, the

degree of effectiveness is broadly comparable with the results of large-scale cohort studies carried out annually by the Japan Physicians Association, which indicate that classical influenza vaccination of the over 65s has generally provided only moderate benefit to this age group.[17] In the United States, the Centers for Disease Control estimates that vaccination of the elderly can be 30–70% effective.[18] Although this refers to the prevention of hospitalization rather than the disease itself, our figure of 32·1% lies at the lower end of this range. Vaccine effectiveness in any given year is influenced by how well the strains used in the seasonal vaccine antigenically match those circulating in the community.[19] In 2008–2009, the vaccine and circulating A/H1N1 subtype matched (A/Brisbane/59/2007); the A/H3N2 subtype matched in the first part of the season (A/Uruguay/716/2007), but there was a mismatch from March when A/Perth/16/2009 began circulating. In the case of type B, there was also a mismatch between the vaccine strain and the dominant circulating strain.[20]

Seasonal influenza vaccination is recommended for the elderly in Japan[21] and is available at a subsidized cost,[22] and the benefits are mostly seen in factors such as reduced hospitalization, fewer complications, and lower mortality.[2,23,24] However, the evidence for seasonal vaccination in reducing the incidence of influenza in this age group remains weak,[25] indicating a need for improved vaccines.

The study had some limitations. First, although the participants were living in the community, they were recruited solely from among clinic attendees and may therefore not be representative of the Japanese over-50

population as a whole in terms of their general health. In fact, baseline data showed that nearly half of the subjects (43·6%) had an underlying medical condition, even though more than half were aged under 65. Second, our estimate of TIV effectiveness based on a test-negative case–control analysis needs to be treated with some caution because of possible overestimation[26] and lack of adjustment for confounders and limited sample size. Nevertheless, baseline comparison of the influenza-positive and influenza-negative groups showed that overall the two groups were very similar. Furthermore, information/recall bias was minimized by ensuring that participants were able to keep track of the study variables by means of the prospective diary they were provided.

In summary, these results indicate that influenza is an important cause of ARI leading to higher socioeconomic outcomes and more severe symptoms than other viral ARI. Although clinical effectiveness of neuraminidase inhibitors has been reported, burden of influenza is significant. Current TIV vaccines may not offer an effective prevention against influenza in the elderly population. Continuing research and development toward improving influenza vaccines may play a vital part in reducing the clinical and socioeconomic outcomes of the influenza illness for a growing older population.

Acknowledgements

Conflicts of interest

HI has received funding from Daiichi Sankyo, Toyama Chemical, GSK, Taisho Toyama Pharmaceutical Company, Chugai Pharmaceutical Company for his scientific researches, expert testimony, lectures and travel for academic presentation. Authors YT and MR are employed by GlaxoSmithKline. YT owns stock in GSK.

Funding

References

1 Nichol KL, D'Heilly SJ, Greenberg ME, Ehlinger E. Burden of influenza-like illness and effectiveness of influenza vaccination among working adults aged 50–64 years. Clin Infect Dis 2009; 48:292–298.
2 Fleming DM, Elliot AJ. The impact of influenza on the health and health care utilisation of elderly people. Vaccine 2005; 23(Suppl 1):S1–S9.
3 Thompson WW, Shay DK, Weintraub E *et al.* Influenza-associated hospitalizations in the United States. JAMA 2004; 292:1333–1340.
4 Takahashi M, Nagai M. [Estimation of excess mortality associated with influenza epidemics specific for sex, age and cause of death in Japan during 1987–2005]. Nihon Eiseigaku Zasshi. 2008; 63:5–19. Japanese.
5 Ministry of Internal Affairs and Communications (Japan); Statistics Handbook of Japan 2009: Chapter 2: Population. Statistics Bureau; 2009.
6 UN [Internet]. New York: Demographic Profile of the Older Population. Department of Economic and Social Affairs, Population Division. Report on World Population Ageing: 1950–2050, prepared for the 2002 World Assembly on Ageing. Available at http://www.un.org/esa/population/publications/worldageing19502050/ (Accessed 13 August 2011).
7 ECDC [Internet]. Stockholm: Influenza case definitions. Available at http://ecdc.europa.eu/en/activities/surveillance/EISN/surveillance/Pages/influenza_case_definitions.aspx (Accessed 13 August 2011).
8 Stockton J, Ellis JS, Saville M, Clewley JP, Zambon MC. Multiplex PCR for typing and subtyping influenza and respiratory syncytial viruses. J Clin Microbiol 1998; 36:2990–2995.
9 Falsey AR, Formica MA, Walsh EE. Diagnosis of respiratory syncytial virus infection: comparison of reverse transcription-PCR to viral culture and serology in adults with respiratory illness. J Clin Microbiol 2002; 40:817–820.
10 Peret TC, Boivin G, Li Y *et al.* Characterization of human metapneumoviruses isolated from patients in North America. J Infect Dis 2002; 185:1660–1663. Epub 2002 May 3.
11 Nicholson KG, Kent J, Hammersley V, Cancio E. Acute viral infections of upper respiratory tract in elderly people living in the community: comparative, prospective, population based study of disease burden. BMJ, 1997; 315:1060–1064.
12 Wallace LA, Collins TC, Douglas JD, McIntyre S, Millar J, Carman WF. Virological surveillance of influenza-like illness in the community using PCR and serology. J Clin Virol 2004; 31:40–45.
13 Louie JK, Hacker JK, Gonzales R *et al.* Characterization of viral agents causing acute respiratory infection in a San Francisco University Medical Center Clinic during the influenza season. Clin Infect Dis 2005; 41:822–828. Epub 2005 Aug 4.
14 Ikematsu H. [Clinical characteristic of the elderly in influenza infection]. Nihon Rinsho 2003; 61:1926–1930. Japanese.
15 Potter CW. A history of influenza. J Appl Microbiol 2001; 91:572–579.
16 Orenstein EW, De Serres G, Haber MJ *et al.* Methodologic issues regarding the use of three observational study designs to assess influenza vaccine effectiveness. Int J Epidemiol 2007; 36:623–631. Epub 2007 Apr 2.
17 Kashiwagi S, Kawai N, Ikematsu H, Hirotsu N, Iwaki N. Influenza vaccine in the elderly. Jap J Clin Exp Med 2007; 84:39–41. Japanese.

18 CDC [Internet]. Atlanta: Flu Vaccine Effectiveness: Questions and Answers for Health Professionals. Centers for Disease Control and Prevention. [2009] Available at http://www.cdc.gov/flu/professionals/vaccination/effectivenessqa.htm (Accessed 13 August 2011).

19 Hirota Y, Fukushima W, Fujieda M, Ohfuji S, Maeda A. Essential tools for assessing influenza vaccine efficacy in improperly conducted studies: a Japanese perspective. Vaccine 2008; 26:6455–6458. Epub 2008 Jun 23.

20 IDSC [Internet]. Tokyo: Infectious Disease Surveillance Center; c2004. Available at http://idsc.nih.go.jp (Accessed 13 August 2011).

21 Ozasa K, Kawahito Y, Doi T et al. Retrospective assessment of influenza vaccine effectiveness among the non-institutionalized elderly population in Japan. Vaccine 2006; 24:2537–2543. Epub 2005 Dec 27.

22 Hirota Y, Kaji M. History of influenza vaccination programs in Japan. Vaccine 2008; 26:6451–6454. Epub 2008 Jun 23.

23 Gross PA, Hermogenes AW, Sacks HS, Lau J, Levandowski RA. The efficacy of influenza vaccine in elderly persons. A meta-analysis and review of the literature. Ann Intern Med 1995; 123:518–527.

24 Kobayashi K, Washio M, Sakauchi F et al. Efficacy of influenza vaccine in reducing hospital admissions among elderly nursing home residents in winter: the Hokkaido influenza study. Int Med J 2005; 12:89–92.

25 Jefferson T, Di Pietrantonj C, Al-Ansary LA, Ferroni E, Thorning S, Thomas RE. Vaccines for preventing influenza in the elderly. Cochrane Database Syst Rev 2010; DOI: 10.1002/14651858.CD004876.pub3.

26 Belongia EA, Kieke BA, Donahue JG et al. Effectiveness of inactivated influenza vaccines varied substantially with antigenic match from the 2004–2005 season to the 2006–2007 season. J Infect Dis 2009; 199:159–167.