Table 1
Influenza vaccination and attack rates of influenza-like illness (ILI) among school children in the 1984–1985 season (Maebashi Study)

City	Vaccination	Number of subjects (distribution%)	Number of ILIs (attack rate%)
Cities with no mass vaccination			
Maebashi	Total	25,122 (100)	10,743 (42.8)[a]
	Non-vaccinees	25,101 (99.9)	10,738 (42.8)
	Once-vaccinees	18 (0.1)	5 (27.8)
	Twice-vaccinees	3 (0.0)	0 (0)
Annaka	Total	4,021 (100)	1,832 (45.6)
	Non-vaccinees	4,021 (100)	1,832 (45.6)
	Once-vaccinees	0 (0)	0 (0)
	Twice-vaccinees	0 (0)	0 (0)
Cities with mass vaccination			
Total	Total	45,336 (100)	19,817 (43.7)
	Non-vaccinees	7,241 (16.0)	3,962 (54.7)[b]
	Once-vaccinees	5,445 (12.0)	2,603 (47.8)
	Twice-vaccinees	32,641 (72.0)	13,255 (40.6)[a,b]
Takasaki	Total	22,119 (100)	8,865 (40.1)
	Non-vaccinees	1,887 (8.5)	1,017 (53.9)
	Once-vaccinees	1,291 (5.8)	597 (45.9)
	Twice-vaccinees	18,941 (85.6)	7,254 (38.3)
Kiryu	Total	12,374 (100)	5,324 (43.0)
	Non-vaccinees	2,751 (22.2)	1,425 (51.8)
	Once-vaccinees	2,318 (18.7)	1,039 (44.8)
	Twice-vaccinees	7,305 (59.0)	2,860 (39.2)
Isesaki	Total	10,843 (100)	5,628 (51.9)
	Non-vaccinees	2,603 (24.0)	1,520 (58.4)
	Once-vaccinees	1,836 (16.9)	967 (52.7)
	Twice-vaccinees	6,395 (59.0)	3,141 (49.1)

ILI: "fever ≥ 37 °C plus absenteeism ≥ 2 consecutive days" or "absenteeism ≥ 3 consecutive days." Observations from January 8, 1985 to February 28, 1985.
[a] Compared in the second analysis.
[b] Compared in the first analysis.

Table 2
Influenza vaccination and attack rates of influenza-like illness (ILI) among school children in the 1985–1986 season (Maebashi Study)

City	Vaccination	Number of subjects (distribution%)	Number of ILIs (attack rate%)
Cities with no mass vaccination			
Maebashi	Total	24,266 (100)	6,714 (27.7)[a]
	Non-vaccinees	24,249 (99.0)	6,709 (27.7)
	Once-vaccinees	10 (0.0)	5 (50.0)
	Twice-vaccinees	7 (0.0)	0 (0)
Annaka	Total	4,071 (100)	903 (22.2)
	Non-vaccinees	4,056 (99.6)	899 (22.2)
	Once-vaccinees	11 (0.3)	3 (27.3)
	Twice-vaccinees	4 (0.1)	1 (25.0)
Cities with mass vaccination			
Total	Total	43,687 (100)	10,513 (24.1)
	Non-vaccinees	7,702 (17.6)	2,564 (33.3)[b]
	Once-vaccinees	7,778 (17.8)	2,220 (28.5)
	Twice-vaccinees	28,207 (64.6)	5,729 (20.3)[a,b]
Takasaki	Total	21,381 (100)	4,481 (21.0)
	Non-vaccinees	2,063 (9.6)	637 (30.9)
	Once-vaccinees	2,106 (9.8)	640 (30.4)
	Twice-vaccinees	17,212 (80.5)	3,204 (18.6)
Kiryu	Total	11,657 (100)	2,933 (25.2)
	Non-vaccinees	2,628 (22.5)	846 (32.2)
	Once-vaccinees	3,470 (29.8)	817 (23.5)
	Twice-vaccinees	5,559 (47.7)	1,270 (22.8)
Isesaki	Total	10,649 (100)	3,099 (29.1)
	Non-vaccinees	3,011 (28.3)	1,081 (35.9)
	Once-vaccinees	2,202 (20.7)	763 (34.7)
	Twice-vaccinees	5,436 (51.0)	1,255 (23.1)

ILI: same as Table 1. Observations from November 3, 1985 to December 28, 1985.
[a] Compared in the second analysis.
[b] Compared in the first analysis.

dent attitude in interpreting the results. On the other hand, many so-called influenza specialists, who misunderstand the Maebashi Study based on the mass media information, simply believe that the study raised doubts about influenza vaccine efficacy. Needless to say, they have not delved into the details of the study itself, nor have they noticed its important message on case definition, confounding, selection bias, surveillance with equal intensity, among others.

The ecological fallacy that has been intentionally drawn from the data in the Maebashi Study has been spread by the anti-vaccination campaign. Currently, only a few influenza specialists can differentiate between inferences drawn from an ecological study and those drawn from ordinary, analytical epidemiological studies. Influenza specialists involved in vaccination programs should be expected to acquire such basic knowledge and to use it to correctly inform the general public about the propaganda against the influenza vaccine. Influenza specialists, both in Japan and in other countries, must understand the advantages and disadvantages of ecological studies, in the light of recent ecological studies and their impact [2,3].

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Selection bias in evaluating of influenza vaccine effectiveness: A lesson from an observational study of elderly nursing home residents

Wakaba Fukushima[a,*], Yoshimitsu Hayashi[b], Yaichi Mizuno[b], Kanzo Suzuki[c], Tetsuo Kase[d], Satoko Ohfuji[a], Megumi Fujieda[a], Akiko Maeda[a], Yoshio Hirota[a]

[a] Department of Public Health, Osaka City University Faculty of Medicine, 1-4-3, Asahi-machi, Abeno-ku, Osaka 545-8585, Japan
[b] Nagoya-shi Kouseiin Medical Welfare Center, 1501, Sekobo 2-chome, Meito-ku, Nagoya city, Aichi 465-8610, Japan
[c] City of Nagoya, Minato Public Health Center, 2-2-1, Kouei, Minato-ku, Nagoya city, Aichi 455-0015, Japan
[d] Department of Infectious Diseases, Osaka Prefectural Institute of Public Health, 1-3-69, Nakamichi, Higashinari-ku, Osaka 537-0025, Japan

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ABSTRACT

Selection bias is of critical concern in the study of influenza vaccine effectiveness when using an observational study design. This bias is attributable to the inherently different characteristics between vaccinees and non-vaccinees. The differences, which are related both to vaccination and signs of clinical disease as an outcome, may lead to erroneous estimation of the effectiveness. In this report, we describe how selection bias among elderly nursing home residents may lead to a spurious interpretation of the protective effect of influenza vaccine. Our results should be a lesson in the importance of regarding selection bias when assessing influenza vaccine effectiveness.

1. Introduction

Selection bias is a kind of systematic error in epidemiologic studies. The common element of selection bias is that the relationship between exposure and disease is different between participants and non-participants in the study. As an example, one may imagine "self-selection bias" or "healthy worker effect" [1]. Using the study of influenza vaccine effectiveness, we adopt influenza-like illness (ILI) as an outcome measure. The inherently different characteristics between vaccinated and non-vaccinated group may be obvious. Specifically, vaccinees may be health-conscious and have behavior that decreases the risk of ILI. Therefore, the vaccinated group might be expected to have a lower rate of ILI for reasons that are unrelated to the vaccination. The opposite assumption is that the vaccinees may have underlying disease that predisposes to a higher rate of ILI, and puts them in a higher risk group than non-vaccinees. The different characteristics between the groups, as related both to vaccination and the clinical disease status as an outcome, may lead to erroneous estimation of the vaccine effectiveness.

Such a systematic error is regarded as confounding if distortion due to the error is able to be accurately controlled for in the analysis. However, since variables potentially confounding the association of interest are not always possible to identify and quantify, this error may exert unpredictable effect as selection bias [2]. In general, the preferred epidemiologic study design for minimizing the extent of selection bias in assessing vaccine is the randomized controlled trial [3]. Nevertheless, specific characteristics of naturally occurring seasonal influenza epidemics make it difficult to obtain conclusive findings from a single field trial. Namely, the unpredictable nature of time and intensity of the influenza occurrence, the presence of different virus strains in an epidemic, and antigenic differences between the vaccine strains and epidemic viruses, are all factors that can affect the outcome of a vaccine trial [4]. Taken together with the recent ethical concerns regarding the use of placebo in vaccine trials, cost issues, and logistic problems, it is now the norm to use a non-experimental study design [5]. Although several methods have been proposed to address the issues of systematic errors [5–7], no definitive resolution has emerged.

In this report, we present an experience from a prospective cohort study to evaluate the influenza vaccine effectiveness in elderly nursing home residents. In our study, we detected possible spurious protective effects due to selection bias. By estimating vaccine effectiveness during different periods of the influenza season, we became aware of the existence of the bias.

2. Materials and methods

All residents in an elderly nursing home, located in Nagoya City, Japan, as of 1 December 2003 were included in this study.

Abbreviations: OR, odds ratio; CI, confidence interval; ILI, influenza-like illness.

* Corresponding author. Tel.: +81 6 6645 3756; fax: +81 6 6645 3757.
E-mail address: wakaba@med.osaka-cu.ac.jp (W. Fukushima).

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Table 1
Baseline characteristics of vaccinees and non-vaccinees

Variable	Vaccinees (N = 166)	Non-vaccinees (N = 118)	P-value[a]
Sex (male)	49(30)	36(31)	0.858
Age (years): median (25, 75 percentile)	84(77,90)	86(78,92)	0.344[b]
Smoking (yes)	10(6)	7(6)	0.974
Heart disease (yes)	101(61)	77(65)	0.449
Lung disease (yes)	42(25)	28(24)	0.762
Cerebro-vascular disease (yes)	98(59)	66(56)	0.602
Diabetes mellitus (yes)	28(17)	17(14)	0.576
Hypertension (yes)	83(50)	55(47)	0.573
Steroid/immnosuppesunt use (yes)	3(2)	4(3)	0.455
Functional status (bedridden)	107(64)	96(81)	0.002
Dementia (required assistance)	74(45)	75(64)	0.002
Albumin level (<3.8 g/dl)	106(64)	99(84)	0.0002

Note: The distribution of subjects by vaccination status is expressed as number and percentage in parenthesis, otherwise indicated.
[a] Chi square test or Fisher exact test, except for age.
[b] Wilcoxon rank sum test.

A total of 284 residents (mean age, 85 years; 85 men, 199 women) were enrolled. Based on institutional policy, only residents who were able to provide informed consent could receive the vaccine. Informed consent provided by family members was not acceptable. Vaccination with a commercial inactivated influenza vaccine was done in 166/284 residents. A 0.5 ml dose contained 15 μg each of A/New Caledonia/20/99(H1N1), A/Panama/2007/99(H3N2), and B/Shandong/7/97 antigens. Subcutaneous injections of 0.5 ml were given once before the influenza season.

For baseline data, we collected information from medical records on demographic characteristics, smoking status, underlying medical conditions (heart disease, lung disease, cerebrovascular disease, diabetes mellitus, and hypertension), steroid/immunosuppressant use, functional status (bedridden or not), dementia (required assistance or not), and clinical laboratory results. The defined outcomes in the study were febrile illnesses of ≥38 °C and ≥39 °C, pneumonia, and death by all causes.

During the typical 17-week influenza season, from 1 December 2003 until 28 March 2004, specifically from the 49th week of 2003 to the 13th week of 2004, we prospectively surveyed the weekly occurrence of each outcome. For febrile illness, the highest temperature during a week was recorded. The physician in charge took throat swabs from residents whenever influenza was suspected and swabs were tested immediately using a rapid antigen test (Capilia Flu A + B, Nippon Becton Dickinson Company, Ltd.). If the result was positive, viral cultures were done. The study protocol was approved by the ethics committee at the Osaka City University Graduate School of Medicine.

SAS Version 8.2 (SAS Institute, Inc., Cary, North Carolina) was used for statistical analyses. Logistic regression was used to estimate vaccine effectiveness by calculating adjusted odds ratios (ORs) and 95% confidence intervals (95% CIs). All explanatory variables, except for age, were dichotomized when included in the model. To account for the time necessary for the vaccine to induce effective antibody, outcomes that occurred within 14 days after vaccination were excluded. All P values are two-sided, the level of significance was 5%, and a value of $0.05 < P < 0.1$ was considered as marginally significant.

3. Results

All participants were followed until either the end of the 2003–2004 influenza season or death, if it occurred during the study period. An influenza outbreak in the nursing home was not virologically confirmed since none of the patient samples were positive by the influenza viral antigen rapid test.

Baseline characteristics between vaccinated and non-vaccinated group were documented (Table 1). A significantly higher proportion of non-vaccinees were bedridden or had dementia that required assistance as compared to vaccinees (81% vs. 64%, $P = 0.002$; and 64% vs. 45%, $P = 0.002$, respectively). A significantly higher proportion of non-vaccinees had lower serum albumin levels (84% vs. 64%, $P = 0.0002$) than vaccinees. There were no significant differences between groups for any of the other variables studied.

The vaccine effectiveness for each of four outcomes was evaluated (Table 2). Based on the observations made during the entire 17-week influenza season, the multivariate analysis revealed that vaccination was effective at preventing febrile illness of ≥38 °C (adjusted OR, 0.48; 95% CI, 0.26–0.90; $P = 0.022$) and ≥39 °C (OR, 0.44; 95% CI 0.17–1.14; $P = 0.091$), and all causes of death (OR, 0.37; 95% CI, 0.12–1.15; $P = 0.084$) with at least marginal significance. The adjusted OR did not reach statistical significance ($P = 0.121$) for an association with preventing pneumonia, but the point estimate of the OR (0.42) suggested a protective effect.

We performed an additional analysis to detect if vaccine effectiveness was more pronounced [8,9] when observations were confined to the intensive influenza season. The two types of epidemic curves representing the reported number of clinically diagnosed influenza cases per sentinel from 70 surveillance sites in the community, and the number of residents with febrile illness of ≥38 °C in the nursing home are shown in Fig. 1. In the community,

Table 2
Outcome occurrence and corresponding vaccine effectiveness[a]

Outcome	Vaccinees (N = 166), n(%)	Non-vaccinees (N = 118), n(%)	Crude OR (95%CI)	P-value	Adjusted OR[b] (95%CI)	P-value
All febrile illness (≥38 °C)	29(17)	38(32)	0.45 (0.26–0.78)	0.004	0.48 (0.26–0.90)	0.022
All febrile illness (≥39 °C)	9(5)	14(12)	0.43 (0.18–1.02)	0.055	0.44 (0.17–1.14)	0.091
Pneumonia	6(4)	11(9)	0.37 (0.13–1.02)	0.054	0.42 (0.14–1.26)	0.121
All causes of death	6(4)	14(12)	0.28 (0.10–0.75)	0.011	0.37 (0.12–1.15)	0.084

[a] Analysis during the entire 17-week influenza season from the 49th week of 2003 to the 13th week of 2004.
[b] Adjusted for sex, age (continuous variable), smoking status, underlying medical conditions (heart disease, lung disease, cerebrovascular disease, diabetes mellitus, and hypertension), steroid/immunosuppressant use, functional status (bedridden or not), dementia (required assistance or not), and albumin level (<3.8 or ≥3.8 mg/dl).

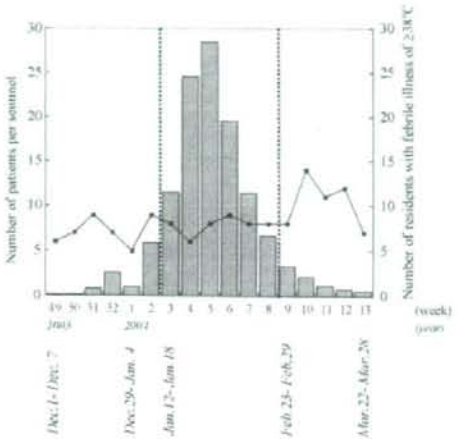

Fig. 1. Changes in the number of clinically diagnosed influenza patients per sentinel reported weekly from 70 surveillance sites in Nagoya city (bar), and the number of residents with febrile illness of ≥38 °C in the nursing home (line) during the 2003–2004 season.

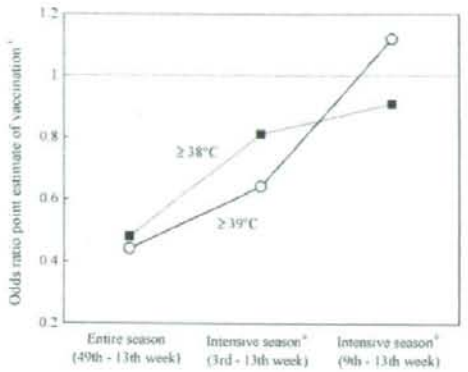

Fig. 2. Change of odds ratio point estimate of vaccination for all febrile illness of ≥38 °C (square) and ≥39 °C (circle) during three different periods of the season. [a] Period that the reported number of clinically diagnosed influenza patients per sentinel was 10 or more persons, based on the weekly report from 70 surveillance sites in Nagoya city during the 2003–2004 influenza season. [b] Period defined as a small peak in a fever curve which presented the number of residents with febrile illness of ≥38 °C in the nursing home. [c] Adjusted for sex, age (continuous variable), smoking status, underlying medical conditions (heart disease, lung disease, cerebrovascular disease, diabetes mellitus, and hypertension), steroid/immunosuppressant use, functional status (bedridden or not), dementia (required assistance or not), and albumin level (<3.8 or ≥3.8 mg/dl).

there were less than 10 patients per sentinel until the 2nd week of 2004. In the nursing home, a small peak appeared after the 9th week in 2004. We interpreted this data as meaning that the peak of the influenza epidemic in the community started from the 3rd week of 2004 on 12 January and that the period following 11 weeks represented the intensive influenza season. Furthermore, from the 9th week of 2004, beginning on 23 February, a second period of the intensive influenza season was noted.

Table 3 shows the outcome occurrence between vaccinated and non-vaccinated groups during the entire season, as well as for the two different periods of the intensive influenza seasons. Based on the comparisons made during the entire season, a significantly higher proportion of non-vaccinees had febrile illness as compared to those receiving vaccine ($P = 0.004–0.049$). However, such differences became smaller and lost statistical significance as we limited the comparisons to the later periods of the season.

The corresponding adjusted odds ratios were calculated during three different periods of the season (Fig. 2). Since there were very few subjects with pneumonia or death, especially in the later period of the season, only febrile illness was considered in the multivariate analysis. Both ORs for febrile illness of ≥38 °C and ≥39 °C were shifted toward the null value, indicating no effect, later in the influenza season.

4. Discussion

In this study, there are different baseline characteristics between vaccinated and non-vaccinated groups. Variables such as being bedridden, having dementia that requires assistance, and lower serum albumin levels, occurred more frequently among non-vaccinees than vaccinees. Functional and nutritional status has been reported as potential confounder in determining either effectiveness or the nature of the immune response of influenza vaccine

Table 3
Outcome occurrence during three different periods of the season

Outcome	Vaccinees ($N = 166$), n (%)	Nonvaccinees ($N = 118$), n (%)	P-value[c]
Entire season (49th–13th week)			
All febrile illness (≥38 °C)	29 (17)	38 (32)	0.004
All febrile illness (≥39 °C)	9 (5)	14 (12)	0.049
Pneumonia	6 (4)	11 (9)	0.046
All causes of death	6 (4)	14 (12)	0.007
Intensive season[a] (3rd–13th week)			
All febrile illness (≥38 °C)	26 (16)	25 (21)	0.232
All febrile illness (≥39 °C)	8 (5)	10 (8)	0.213
Pneumonia	5 (3)	6 (5)	0.534
All causes of death	4 (2)	6 (5)	0.328
Intensive season[b] (9th–13th week)			
All febrile illness (≥38 °C)	16 (10)	14 (12)	0.548
All febrile illness (≥39 °C)	5 (4)	7 (4)	1.000
Pneumonia	2 (2)	3 (2)	1.000
All causes of death	2 (2)	1 (1)	0.572

[a] Period that the reported number of clinically diagnosed influenza patients per sentinel was 10 or more persons, based on the weekly report from 70 surveillance sites in Nagoya city during the 2003–2004 influenza season.
[b] Period defined as a small peak in a fever curve which presented the number of residents with febrile illness of ≥38 °C in the nursing home.
[c] Chi square test or Fisher exact test.

in the elderly [10,11]. Since such differences were likely to contribute to overestimation of vaccine effectiveness, we adjusted for these variables in the analysis [5]. However, adjustment did not significantly reduce systematic error.

Our conclusion that selection bias was the most plausible explanation for the spurious results regarding vaccine was based on the following information. Despite the fact that we did not virologically confirm the influenza outbreak, a protective effect of vaccination was suggested when we considered outcome occurrences during the entire influenza season. Unexpectedly, these effects disappeared when we analyzed the intensive period of the influenza season as a separate entity. Most outcome occurrences among non-vaccinees developed in the initial period. This is likely to be the result of the institutional consenting policy. Since participation was limited to those who were able to give informed consent by themselves, the non-vaccinated group included both individuals too frail to be consented, as well as those who elected not to participate. Those individuals were likely to be at high risk for significant clinical outcomes early in the influenza season.

In addition, a recent study has suggested the influence of bias in study of influenza vaccine effectiveness among elderly [12]. In this study, the relative risk was estimated separately before, during, and after the influenza season. The authors pointed out the movement of the effectiveness of vaccine toward null later in the season, and concluded that relatively healthy seniors prefer to receive vaccine. Although the process to define each period of the season for additional analysis was different from our study, the approach to identify the possible existence of bias was similar.

Alternative explanations for our unexpected findings should also be considered. First, we cannot exclude that an influenza epidemic in the nursing home had occurred before the entire season as defined in this study. If the timing of the outbreak was prior to the season, it would be reasonable to expect that vaccine effectiveness would disappear if the observation period was limited to the later period. However, since nosocomial infectious disease is monitored in the nursing home throughout the year, the assumption of a prior epidemic is unlikely. Second, older persons might have lower post-vaccination antibody titers than healthy young adults [13]. In a randomized trial, vaccine efficacy among persons aged ≥70 years was lower compared with relatively younger seniors [9]. Besides, a major limitation of this study was that non-specific outcomes were adopted as end-points, rather than ILIs, which are usually defined as illnesses with rhinorrhea, sore throat, and/or cough, plus fever [8,14]. This limitation was because many of the patients were bedridden or had dementia that required assistance. Those who were bedridden commonly have rhinorrhea and a cough. It is also difficult for those with dementia to reliably report the presence of sore throat. Thus, to prove vaccine effectiveness among elderly nursing home residents may be complicated.

Despite the limitations, selection bias seems to be a reasonable interpretation for our findings. The key message is the importance of analyzing the data from multiple angles, whether or not positive results are obtained. To estimate vaccine effectiveness during different periods of the season may be the most informative approach. Further exploration and discussion are needed avoid introducing inappropriate inferences in the study of influenza vaccine effectiveness.

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Influenza vaccine effectiveness and confounding factors among young children

Megumi Fujieda[a], Akiko Maeda[a], Kyoko Kondo[a], Wakaba Fukushima[a,*], Satoko Ohfuji[a], Masaro Kaji[b], Yoshio Hirota[a]

[a] Department of Public Health, Osaka City University Faculty of Medicine, Asahi-machi 1-4-3, Abeno-ku, Osaka 545-8585, Japan
[b] Kurume University, Asahi-machi, Kurume-shi 830-0011, Japan

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ABSTRACT

This study, done during the 2002–2003 season among children <6 years of age to investigate influenza vaccine effectiveness and confounding factors, involved 2913 children (1512 vaccinees, 1401 non-vaccinees) recruited from 54 paediatric clinics. Between December 2002 and April 2003, parents reported their children's maximum body temperatures weekly. Influenza-like illness (ILI) was defined as an acute febrile illness (≥38.0 C) during the peak epidemic period. Adjusted odds ratios (ORs) for ILI were obtained using a logistic regression model. In analysis for total subjects, the ORs were significantly decreased for vaccinees (OR: 0.76, 95% CI: 0.66–0.88) and significantly increased for younger age groups, including children aged 2.0–3.9 years (1.42, 1.18–1.72) and those <2.0 years (2.02,1.61–2.54), compared to those between 4.0 and 5.9 years. ORs were significantly increased for children who visited a physician within the last 6 months for a cold (1.27, 1.08–1.50), attended preschool (1.72, 1.45–2.04), and had ≥3 siblings (1.42, 1.15–1.74). These confounding factors are suggested to be considered in estimating vaccine effectiveness among young children. In subgroup analysis by age groups, significantly decreased ORs were seen in 2.0–3.9-year-old (0.59, 0.47–0.74) and 4.0–5.9-year-old (0.75, 0.58–0.98) vaccinees; no significant vaccine effectiveness was detected for those <2.0 years (1.07, 0.80–1.44). Thus, among very young children vaccine effectiveness could not be demonstrated.

1. Introduction

It has been reported that healthy children less than 2 years of age have high influenza related hospitalization rates, that are, in fact, similar to the hospitalization rates among older adults for whom annual influenza vaccination is recommended [1,2]. Recently, the US Advisory Committee on Immunization Practices recommended that, beginning with the 2006–2007 season, influenza vaccine be administered to all children under 5 years of age. This expands on the recommendation to vaccinate only children aged 6–23 months in the previous two seasons [3].

Previous studies have investigated the efficacy of influenza vaccine among young children [4–6], but their results are not consistent. In addition, few studies have investigated the factors that influence influenza vaccine effectiveness among young children. Therefore, this study was conducted during the 2002–2003 season to assess the effectiveness of influenza vaccine and to identify the confounding factors that distort the estimated influenza vaccine effectiveness among children under 6 years of age.

2. Material and methods

Details of the study have been previously described [7]. Briefly, the study subjects were children under 6 years of age who were recruited from 54 paediatric clinics located in 8 different areas of Japan, between October 1, 2002 and December 15, 2002. A total of 2934 children (1521 vaccinees and 1413 non-vaccinees) were enrolled in this study. At each clinic, the children who received vaccine on parental request were entered into the vaccinated group. The unvaccinated group consisted of the one or two children who visited the paediatrician after each vaccinee and whose parents did not request to have their children vaccinated. For vaccinees, two doses of vaccine, containing A/New Caledonia/20/99(H1N1), A/Panama/2007/99(H3N2) and B/Shandong/7/97, were given subcutaneously. Children under 1 year of age received a 0.1 ml dose, while children 1 year of age and older received a 0.2 ml dose.

Physical and environmental data were obtained using self-administered questionnaires completed by parents or guardians at the time of enrolment. The data collected included: date of birth; gestational age; birth weight; preschool attendance; number of family members; number of siblings; number of rooms; total room space of the residence; disease onset during the previous influenza season and history of hospitalization; and influenza vaccinations

* Corresponding author. Tel.: +81 6 6645 3756; fax: +81 6 6645 3757.
E-mail address: wakaba@med.osaka-cu.ac.jp (W. Fukushima).

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within the last 3 years. The information about health-related conditions collected from the children's paediatricians using a structured questionnaire included: vaccination status; vaccine manufacturer and lot number if vaccinated; current body weight; physician visits within the previous 6 months (for cold symptoms, otitis media, digestive symptoms, or other reasons); underlying conditions (nine diseases); and long-term treatment with corticosteroids or aspirin.

With respect to the follow-up survey, the parents were requested to report the child's maximum body temperature every week. This questionnaire was to be returned to the paediatrician's office each week from the 51st week of 2002 to the 15th week of 2003. An influenza-like illness (ILI) was defined as an acute febrile illness that occurred during the highest epidemic period of influenza recorded in each study area. All subjects were classified into 3 categories according to the highest body temperature reported during the peak epidemic period: non-disease with fever <38.0 °C; ILI with fever 38.0–38.9 °C; and ILI with fever ≥39.0 °C.

To compare the characteristics of vaccinees and non-vaccinees, Chi-square or Fisher's exact test, and the Wilcoxon rank-sum test were employed. In order to assess independent associations between each outcome of ILI and the vaccination status or confounding factors, adjusted odds ratios (ORs) and 95% confidence intervals (95% CI) were calculated by a proportional odds model or a binary model on logistic regression. The stepwise method with default P values of 0.05 to enter and remove was used to determine the final model. Vaccine effectiveness was equivalent to $(1 - \mathrm{OR}) \times 100\%$. All reported P values are two-sided. All data analyses were carried out using the SAS statistical software package (Version 9.1; SAS Institute, Inc., Cary, NC, USA).

3. Results

Data from 2913 subjects were analyzed; 21 subjects were excluded from the initial 2934 due to non-participation in the follow-up survey. The mean age of the vaccinees was 3.4 years, and the mean age of the non-vaccinees was 2.8 years. Among the vaccinees, 52% were male; among the non-vaccinees, 53% were male. The baseline characteristics that were statistically significantly different between the two groups are shown in Table 1. Older age, heavier current body weight, smaller family size, fewer siblings, preschool attendance, and previous vaccinations were more frequent in the vaccinated group. The univariate analysis results for all these factors are shown in Table 2.

In the first analysis, crude and adjusted ORs for ILI were calculated using a proportional odds model with a three-level outcome variable (maximum body temperature: <38.0, 38.0–38.9, ≥39.0 °C) (Table 2). Five explanatory variables (vaccination, age, number of siblings, physician visits for cold symptoms within the last 6 months and preschool attendance) were selected. When analyzed as an entire sample, the adjusted OR of vaccinees decreased significantly to 0.76 (95% CI: 0.66–0.88). Thus, vaccine effectiveness was estimated to be 24% (95% CI: 12–34%). Increased adjusted ORs were observed for the younger age groups of <2.0 years (OR: 2.02, 95% CI: 1.61–2.54) and 2.0–3.9 years (OR: 1.42, 1.18–1.72), preschool attendance (OR: 1.72, 1.45–2.04), having 3 or more siblings (OR: 1.42, 1.15–1.74), and physician visits within the last 6 months for a cold (OR: 1.27, 1.08–1.50). Significant dose–response relationships with increasing adjusted ORs were shown for both a decrease in age and an increase in the number of siblings ($P = 0.000$ and 0.001, respectively).

Secondly, same as the first analysis proportional odds model were also used to calculate adjusted ORs by age group (Table 3). Significantly decreased ORs of vaccination were observed for the 2.0–3.9 age group (OR: 0.59, 0.47–0.74) and the 4.0–5.9 age group (OR: 0.75, 0.58–0.98). In contrast, the adjusted OR was 1.07 (0.80–1.44) among those aged <2.0 years; a decreased OR was not observed.

Table 1
Baseline characteristics of the study participants by vaccination status

Characteristics	Vaccinee ($n = 1512$)	Non-vaccinee ($n = 1401$)	P value[a]
Health-related conditions collected from parents or guardians			
Age (years)	3.4	2.8	0.000
Birthweight (%)			
<2000 g	2	1	0.026
2000–2499 g	8	7	
2500–2999 g	40	36	
3000–3499 g	38	42	
3500–3999 g	11	13	
>4000 g	1	1	
Influenza vaccination within last 3 years (%)	70	9	0.000
Disease onset in previous season[b] (%)	42	39	0.044
History of hospitalization (%)	33	23	0.000
Health-related conditions collected from paediatrician			
Current body weight[b] (kg)	14.5	13.1	0.000
Physician visits within last 6 months (%)			
For otitis media	9	7	0.018
For cold symptoms	76	74	0.292
Underlying illnesses (%)			
Atopy	7	11	0.000
Environmental characteristics collected from parents or guardians			
Preschool attendance (%)	62	46	0.000
Number of family members	4.2	4.4	0.000
Number of siblings	1.9	2.0	0.000
Number of rooms	4.4	4.3	0.010
Total room space (m^2)	76.1	69.1	0.000

Except where indicated otherwise, values are mean.
[a] χ^2 test or Wilcoxon rank-sum test.
[b] One subject was excluded as data was missing.

In the third analysis, the ORs were calculated using binary models with combinations of two-level outcomes (<38.0 °C versus ≥38.0 °C, <39.0 °C versus ≥39.0 °C). In these computations, the same explanatory variables as those in the first analysis for total subjects were considered for adjustment. The adjusted ORs of vaccination for all subjects were 0.77 (0.66–0.90) for <38.0 °C versus ≥38.0 °C, and 0.74 (0.62–0.88) for <39.0 °C versus ≥39.0 °C.

In the fourth analysis, adjusted ORs were calculated for age groups comparing <38.0 °C versus ≥38.0 °C and <39.0 °C versus ≥39.0 °C. The adjusted ORs for 2.0–3.9 years were 0.62 (0.48–0.79) for <38.0 °C versus ≥38.0 °C and 0.53 (0.40–0.70) for <39.0 °C versus ≥39.0 °C; for 4.0–5.9 years, the adjusted ORs were 0.75 (0.58–0.99) for <38.0 °C versus ≥38.0 °C and 0.76 (0.55–1.04) for <39.0 °C versus ≥39.0 °C. Even when different outcome definitions were used, significantly or marginally significantly decreased ORs were observed for the older 2 age groups. ORs in the lowest age group were 1.05 (0.76–1.44) for <38.0 °C versus ≥38.0 °C and 1.10 (0.78–1.55) for <39.0 °C versus ≥39.0 °C; decreased ORs were not observed in this age group.

4. Discussion

This study was conducted using a non-randomized design, in which vaccination or non-vaccination was self-selected by the parents. Therefore, it is essential to consider potential confounders that could have been unequally distributed between the groups. In fact, Table 2 shows that vaccine effectiveness in all subjects was

Table 2
Odds ratios for ILI by vaccination status and characteristics of children under 6 years of age, calculated using a proportional odds model with a three-level outcome

Vaccination status and characteristics	Crude OR			Adjusted OR		
	OR	95% CI	*P* value	OR	95% CI	*P* value
Non-vaccinee	1.00			1.00		
Vaccinee	0.73	0.64–0.84	0.000	0.76	0.66–0.88	0.000
Age (years)						
<2.0	1.45	1.21–1.74	0.000	2.02	1.61–2.54	0.000
2.0–3.9	1.09	0.92–1.29	0.307	1.42	1.18–1.72	0.000
4.0–5.9	1.00			1.00		
		Trend $P=0.000$			Trend $P=0.000$	
Birthweight (g)						
<2500	1.00					
2500–2999	1.05	0.81–1.37	0.700			
3000–3499	1.12	0.87–1.45	0.389			
>3500	1.10	0.81–1.50	0.530			
		Trend $P=0.364$				
Influenza vaccination within last 3 years						
No	1.00					
Yes	0.68	0.59–0.79	0.000			
Disease onset in previous season						
No	1.00					
Yes	1.23	1.07–1.42	0.004			
History of hospitalization						
No	1.00					
Yes	1.11	0.95–1.29	0.196			
Current body weight (kg)						
<10.1	1.00					
10.1–12.5	0.86	0.69–1.07	0.178			
12.6–15.7	0.78	0.63–0.96	0.018			
>15.8	0.67	0.55–0.83	0.000			
		Trend $P=0.000$				
Physician visits within last 6 months for otitis media						
No	1.00					
Yes	1.48	1.15–1.90	0.003			
Physician visits within last 6 months for cold						
No	1.00			1.00		
Yes	1.34	1.14–1.58	0.001	1.27	1.08–1.50	0.005
Atopy						
No	1.00					
Yes	1.19	0.93–1.52	0.173			
Preschool attendance						
No	1.00			1.00		
Yes	1.26	1.09–1.45	0.002	1.72	1.45–2.04	0.000
Number of family members						
<4	1.00					
4	0.99	0.83–1.19	0.935			
>5	1.20	0.99–1.45	0.060			
		Trend $P=0.041$				
Number of siblings						
1	1.00			1.00		
2	1.06	0.90–1.26	0.491	1.14	0.96–1.36	0.135
>3	1.37	1.12–1.67	0.002	1.42	1.15–1.74	0.001
		Trend $P=0.003$			Trend $P=0.001$	
Number of rooms						
<4	1.00					
4	0.93	0.78–1.11	0.408			
5–6	0.97	0.81–1.17	0.761			
>7	0.90	0.70–1.16	0.421			
		Trend $P=0.496$				
Total room space (m^2)						
<41.0	1.00					
41.0–57.3	0.95	0.77–1.17	0.628			
57.4–81.9	1.00	0.82–1.23	0.989			
>82.0	0.98	0.81–1.20	0.875			
		Trend $P=0.972$				

Table 3
Odds ratios of vaccination for different outcome definitions of ILI among children under 6 years of age by age group

Age (years)	Proportional model				Binary model (<38.0 °C vs. ≥38.0 °C)				Binary model (<39.0 °C vs. ≥39.0 °C)			
	Crude		Adjusted		Crude		Adjusted		Crude		Adjusted	
	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value	OR (95% CI)	*P* value
0–5.9[a]	0.73 (0.64–0.84)	0.000	0.76 (0.66–0.88)	0.000	0.74 (0.64–0.86)	0.000	0.77 (0.66–0.90)	0.001	0.72 (0.61–0.85)	0.000	0.74 (0.62–0.88)	0.001
<2.0[b]	1.18 (0.89–1.56)	0.255	1.07 (0.80–1.44)	0.653	1.16 (0.86–1.57)	0.323	1.05 (0.76–1.44)	0.772	1.20 (0.87–1.66)	0.271	1.10 (0.78–1.55)	0.582
2.0–3.9[c]	0.60 (0.48–0.75)	0.000	0.59 (0.47–0.74)	0.000	0.63 (0.50–0.79)	0.000	0.62 (0.48–0.79)	0.000	0.54 (0.41–0.70)	0.000	0.53 (0.40–0.70)	0.000
4.0–5.9[c]	0.78 (0.61–1.01)	0.060	0.75 (0.58–0.98)	0.034	0.78 (0.60–1.02)	0.065	0.75 (0.58–0.99)	0.040	0.79 (0.58–1.08)	0.137	0.76 (0.55–1.04)	0.081

Note: The distribution of subjects by body temperature is expressed as number and percentage in parenthesis. OR, Odds ratio; CI, confidence interval.

[a] Explanatory variables: vaccination, age, siblings, physician visits for cold symptoms within the last 6 months and preschool attendance.

[b] Explanatory variables: vaccination, siblings, preschool attendance, vaccine dosage (or age).

[c] Explanatory variables: vaccination, siblings, physician visits for cold symptoms within the last 6 months and preschool attendance.

decreased by 11% (from 27 to 24%) after adjustment, and derived vaccine effectiveness was shifted toward a null value. A previous study suggested the importance of considering confounding effects when estimating influenza vaccine effectiveness. It was found that after adjustment for functional limitation, the OR for death in vaccinated subjects compared to unvaccinated subjects was closer to null (OR: 0.71, 0.47–1.06) than an unadjusted estimate (OR: 0.59, 0.41–0.83) [8]. In a randomized control trial of influenza vaccine efficacy, the characteristics of vaccinees and non-vaccinees were compared; the adjusted relative risks were calculated and compared to unadjusted values, and they were confirmed to be similar [9]. Indeed, particularly in the present non-randomized study, confounding factors that could influence influenza vaccine effectiveness should be considered in analysis.

Four confounding factors were simultaneously considered in our analysis of vaccine effectiveness. These confounding factors also affect as risk factors of ILI. Firstly, younger age was an increased risk of ILI. This indicates a significant change in susceptibility to illness as children age. This result is consistent with a previous study of children aged 6 months to 8 years, which reported that children 6–11 months and 12–23 months of age had a higher risk of ILI than a reference group of 7–8 year olds (adjusted hazard rate ratio: 5.32 (4.51–6.27), 3.68 (3.16–4.27), respectively) [9]. In another study, subjects' age were older than the present study; nevertheless, elementary school children in higher grades had a significantly decreased risk of ILI (mild ILI, OR: 0.4 (0.2–0.9); severe ILI, 0.2 (0.1–0.6)) [10].

Secondly, variables related to viral exposure increased the risk of ILI. Children with more than 3 siblings had an increased risk of ILI (OR: 1.42, 1.15–1.74). Similar findings were reported in a case–control study in elementary schools, where large family size (≥5 members) increased the risk of ILI (OR: 1.93, 95% CI: 1.10–3.37) [10]. Both the number of siblings and the number of family members indicate the degree of crowding present in the family setting, which can be interpreted as the presence of household contacts increases the probability of influenza infection from infected individuals. Our study also shows that preschool attendance increases the probability of such infectious contacts, as shown by an increased risk of ILI for preschool attendance (OR: 1.72, 1.45–2.04).

It has been reported that children with high risk conditions have an increased risk of influenza-associated outpatient visits and hospitalisations [11]. In addition, significantly increased risks of ILI among elementary school children were found for children with easily inflamed tonsils and with a history of physician-diagnosed asthma [10]. In the present study, physician visits for a cold within the last 6 months were associated with an increased risk of ILI, although no association was found between underlying illnesses and ILI. This is perhaps due, in part, to the fact that most subjects were healthy children. However, the increased risk associated with physician visits for a cold prior to the epidemic seasons may indicate an association between children's general health status and ILI.

Four factors (age, sibling number, preschool attendance, and physician visits for a cold within the last 6 months) except for vaccination status were selected for the final model. Since these factors were associated with an increased risk for ILI, the estimated vaccine effectiveness in this study would be distorted unless an adjustment for these factors were made. Although we carefully considered it in our analysis, there is a limitation to this study, as has been discussed elsewhere [7]. In this study, it is inevitable that non-influenzal illnesses were included in the definition of ILI, which would have affected the results. This is due to the definition of ILI as an acute febrile illness reported during an epidemic peak. In order to measure the onset of a febrile illness, information about the maximum body temperature each week was prospectively collected using a

postal questionnaire. This enabled the identification of ILI onset to be done equally for all subjects throughout an epidemic period. The postal questionnaire not only provided a similar recall stimulus to parents in both groups, but also minimized the uncertainty related to the parents' responses. Thus, it is reasonable to assume that the extent to which the ILI category included non-influenzal illnesses, and the extent to which the non-disease category included influenza *per se*, was similar in the vaccinated and non-vaccinated groups. This non-differential misclassification results in an underestimation of vaccine efficacy, but does not affect the validity of the study results.

In the present study, vaccine effectiveness for ILI was found for children under 6 years of age: 24% (12–34%) as a whole. Vaccine effectiveness for 2.0–3.9 years of age and 4.0–5.9 years of age, were 41% (26–53%) and 25% (2–42%), respectively, and were statistically significant, while it was −7% (−44 to 20%) for children <2.0 years without clear vaccine effectiveness. Thus, influenza vaccine effectiveness could not be demonstrated in this age group. In a recent randomized, controlled study of children 6–24 months of age conducted over 2 consecutive influenza seasons, the vaccine efficacy against culture-proven influenza was not consistent between the study seasons (first year: 66% (34–82%), second year: −7% (−247 to 67%)); although it should be noted that the attack rate was quite different for the 2 seasons [5]. A few factors have been identified that help explain the failure to detect vaccine effectiveness in very young children. It was reported that in children <1 year of age, the immune response to influenza vaccine was lower than in those 1–3 years of age [12]. It has also been shown that influenza epidemics often overlap with the circulation of respiratory syncytial virus (RSV) [2], which has a greater health impact in very young children than in older children [13]. An ILI defined to measure vaccine effectiveness is thus diluted more by non-influenzal illnesses among young children than among older children.

In summary, as a whole, a statistically significant protective effect of influenza vaccine against ILI was found. However, vaccine effectiveness was not clearly shown in children under 2 years of age. Further studies using different methods, in different locations, and in different seasons, are needed to clarify the effectiveness of influenza vaccine among young children.

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Japanese Encephalitis Viral Infection Remains Common in Japan

To the Editor:

Japanese encephalitis (JE) virus is a flavivirus that causes about 15,000 deaths in Asia every year. Mosquitoes and swine play important roles in the spread of JE virus in the paradomestic environment, the former as a vector and the latter as an amplifier. In the 1950s, approximately 5000 cases, about 20% fatal, were reported yearly in Japan, but recently fewer than 10 cases are reported each year.

Reasons for the marked decrease in JE in Japan include decreases in the number of rice fields which are habitats of vector mosquitoes (mostly *Culex tritaeniorhynchus*), subsequent decreases in the mosquito population, and increased distance between the habitats of people and swine. However, urban residents are still bitten by mosquitoes during the summer, with the exception of northern Japan. Furthermore, 50–100% of Japanese pigs younger than 8 months have antibodies against the JE virus in summer and autumn, indicating that most Japanese pigs can still be JE virus producers.

The extremely low reported incidence of JE in human patients in Japan, despite the high prevalence of antibodies against the JE virus in swine and continued mosquito bites in humans, constitutes a paradoxical situation. To explain this paradox, we proposed a hypothesis that human JE viral infections still occur, but produce only mild symptoms. To test this hypothesis, we measured titers of the nonstructural protein-1 (NS1) antibody,[1] which increases only with natural JE viral infection, and titers of the HI antibody, which increases with both vaccination and JE viral natural infection, in blood samples obtained from 50 volunteers and patients, aged 1–88 years, at Eijudo Clinic, located in an urban area of eastern To-

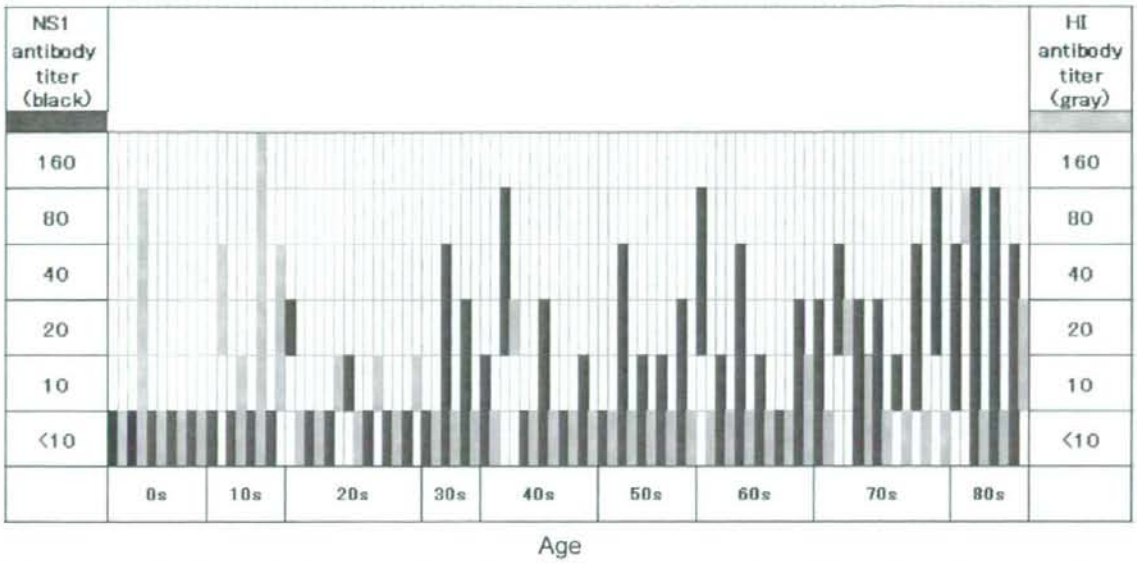

FIGURE 1. Range of NS1 and HI antibody titers against Japanese encephalitis. The black bars indicate changes in NS1 antibody titers in each serum series, and the grey bars show changes in HI antibody concentrations. The adjacent bars are results for a single person. Sera collected from each person for 2–3 years.

kyo. Blood samples were collected for 2–3 years from each subject.

Results are shown in Figure 1. The black bars indicate changes in NS1 antibody titers in each serum series, and the gray bars show changes in HI antibody concentration. The adjacent bars are results for a single person. The bars are arranged horizontally by subject age. The NS1 titers indicate that the incidence of JE viral infection has increased with subject age, whereas HI titers, which reflect vaccination, have slowly fallen with subject age. Two of 50 subjects showed rapid and marked increases in the NS1 titer. One was a 57-year-old woman who took a 3-day trip from June 30 through July 2, 2002, to Okinawa, a southern Japanese island where 3 American soldiers contracted JE with severe sequelae several years ago.[2] The NS1 titer on June 21 was <1:10 but by July 21 had increased to 1:20 and remained high for 1 year. During this time the subject exhibited no symptoms. Another subject was a 85-year-old woman who had remained at home for several years. The NS1 titer was <1:10 on July 8, 2002, but the titers on July 15 and September 8, 2002, were 1:80. From July 8 through 15 she had a low-grade fever, but no infectious focus was identified.

We have shown rising NS1 antibody titers with subject age in a series of blood samples obtained at a clinic in eastern Tokyo and described 2 subjects with rapidly increasing NS1 titers. These results and a previous report[3,4] suggest that human JE viral infection remains prevalent in Japan, although symptoms can be mild or absent. Therefore, vaccination against JE remains extremely important, although the Japanese Government halted strong recommendation of routine JE vaccination in 2005.[5]

Teiichi Matsunaga, MD, PhD
Eijudo Clinic
Katsushika-ku, Tokyo, Japan

Mizue Shoda, MSc
Eiji Konishi, PhD
Department of International Health
Kobe Univ. Graduate Sch. of Health Sciences
Kobe, Japan

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The incidence of pediatric invasive pneumococcal disease in Chiba prefecture, Japan (2003–2005)

Naruhiko Ishiwada [a,*], Tomomichi Kurosaki [b], Itaru Terashima [c], Yoichi Kohno [a]

[a] *Department of Pediatrics, Chiba University, Graduate School of Medicine, Chiba 260-8677, Japan*
[b] *Department of Pediatrics, Chiba Municipal Kaihin Hospital, Chiba 261-0012 Japan*
[c] *Department of Pediatrics, Saiseikai Narashino Hospital, Narashino 275-8580 Japan*

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Summary *Objectives:* The purpose of the study is to evaluate the incidence, spectrum of clinical manifestations and outcome of invasive pneumococcal disease (IPD) in children in Chiba prefecture, Japan.
Methods: To determine the precise incidence of IPD in Chiba prefecture, we implemented a retrospective survey of the period from 2003 to 2005. A written questionnaire was sent to 45 hospitals that have pediatric wards, and information was obtained from all hospitals. The questionnaire included the clinical diagnosis, patient's age, underlying disease, prognosis and antimicrobial susceptibility of the isolated strains.
Results: During the 3 study years, 130 patients were diagnosed with IPD. The mean annual incidence rates of IPD among children <2 and <5 years were 19.5–23.8 and 12.6–13.8 per 100,000, respectively. Among 130 patients with systemic infection, 66 patients had bacteremia, 39 had pneumonia and 16 had meningitis. Five patients had neurological sequelae and 2 patients died. Seventy-four out of 115 isolates (64.3%) exhibited resistance to penicillin G.
Conclusions: The annual incidence of pediatric IPD has remained constant during the study period. Two-third of isolated strains were at least partially resistant to penicillin G. Establishment of appropriate antibiotic therapy against IPD due to penicillin-resistant strains and the introduction of pneumococcal conjugate vaccines are emergent issues in Japan.

Introduction

Streptococcus pneumoniae is one of the leading causes of serious invasive infection, with high mortality and morbidity in children, due to meningitis, septicemia and pneumonia. Over the past decades, the incidence of serious infections due to strains of *S. pneumoniae* with decreased susceptibility to

* Corresponding author. 1-8-1 Inohana, Chuo-ku, Chiba-shi, Chiba-ken, Japan 260-8677. Tel.: +81 43 226 2144; fax: +81 43 226 2145.
E-mail address: ishiwada@faculty.chiba-u.jp (N. Ishiwada).

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penicillin G and broad-spectrum cephalosporin has been steadily increasing worldwide.[1] The number of cases of antibiotic-resistant invasive pneumococcal disease (IPD) has also increased, along with an increasing number of antimicrobial-resistant pneumococcal strains.[2] The emergence of these strains has made the selection of antibiotics for the treatment of IPD more difficult. Recently, the introduction of pneumococcal conjugate vaccines has dramatically decreased the incidence of IPD including antibiotic-nonsusceptible IPD in the United States.[3,4] Accurate up-to-date information on the incidence of IPD is critical to assist decision making for the introduction of pneumococcal vaccines that are appropriate to the country. However, there is little information regarding the incidence of IPD in Japan and the pneumococcal conjugate vaccine has not yet been introduced in Japan. The purpose of this study was to clarify the incidence of IPD in children in the prefecture of Chiba, Japan, using population-based surveillance.

Subjects and methods

To determine the precise incidence of IPD in Chiba prefecture in Japan, we implemented a retrospective survey for the period from 2003 to 2005. Chiba prefecture is one of the 47 prefectures in Japan and is located in the middle of Japan. The population in Chiba prefecture is about 6 million, which represents about 5% of the population of Japan. The population of children less than 2 years of age in Chiba prefecture in 2003, 2004 and 2005 was 109,448, 107,806 and 106,017, respectively. The population of children less than 5 years of age in 2003, 2004 and 2005 was 278,280, 276,108 and 273,468, respectively.[5–7]

A written questionnaire was sent to 45 hospitals in Chiba prefecture that have pediatric wards, and information was obtained from all 45 hospitals either from the clinical records or laboratory records.

IPD was defined as isolation of *S. pneumoniae* from blood cultures in patients with or without focus of infection. The positive rate of blood culture in each hospital was not clarified in this study. The questionnaire included the clinical diagnosis, patient's age, underlying disease, prognosis and antimicrobial susceptibility of the isolated *S. pneumoniae* strains.

Results

During the 3 study years, 130 patients were diagnosed with IPD. The number of cases of IPD in 2003, 2004 and 2005 were 36, 47 and 47, respectively. Among 130 patients with IPD, 66 (50.8%) patients had bacteremia without a focus, 39 had pneumonia, 16 had meningitis and 5 had cellulitis. The mean annual incidence rates of IPD among children less than 2 years of age and less than 5 years of age were 19.5–23.8 and 12.6–13.8 per 100,000, respectively (Table 1). The ages at the onset of IPD were available in 124 patients; 71 (57.3%) in the 0–1-year-old subgroup, 41 (33.1%) in the 2–4-year-old subgroup, 12 (9.7%) in the 5 years or older subgroup. Of the 16 patients with meningitis, 10 (62.5%) were in the 0–1-year-old subgroup, 2 (12.5%) were in the 2–4-year-old subgroup and remaining 4 (25.0%) were in the 5 years or older subgroup (Fig. 1). Fifty-eight percent of the subjects (72/125) were male. At least one underlying condition was documented in 27 (21.8%) of 125 patients with IPD. These included congenital anomaly/syndrome ($n = 6$), bronchial asthma ($n = 4$), malignancy ($n = 4$), congenital heart disease ($n = 3$), neurological disorder ($n = 3$) and others ($n = 7$) in children with IPD. Of the 125 study patients, 5 patients (4%) developed permanent neurological complications. All the patients with neurological sequelae had meningitis. Two study patients (1.6%) died, of which one was aged 3 years with congenital heart disease and the other was aged 17 years with a brain tumor. These fatal cases were diagnosed with fulminant sepsis and pneumonia, respectively. The precise antimicrobial susceptibility of the isolated *S. pneumoniae* strains was known for 115 strains (115/130; 88.5%). Minimal inhibitory concentrations of penicillin G against these 115 *S. pneumoniae* strains were determined by micro-broth dilution method. Of 115 isolates, 49 isolates (42.6%) exhibited intermediate penicillin G resistance and 25 (21.7%) were penicillin G resistant (Fig. 2).

Discussion

Using hospitalized population-based surveillance, this study determined that the annual incidence of IPD among Japanese children younger than 2 and younger than 5 years

Table 1 Annual incidence of invasive pneumococcal infections by clinical diagnosis in Chiba prefecture, Japan

	Number of cases/ 3 years	2003		2004		2005	
		Annual incidence[a]	Annual incidence[b]	Annual incidence[a]	Annual incidence[b]	Annual incidence[a]	Annual incidence[b]
Total IPD	130	23.8	12.6	19.5	13.8	22.6	13.5
Bacteremia	66	13.7	7.2	8.3	7.6	10.4	7.3
Pneumonia	39	3.7	2.2	8.3	4.7	9.4	5.5
Meningitis	16	4.6	2.2	1.9	1.1	2.8	1.1
Cellulitis	5	1.8	0.7	1.9	0.7	0	0.4
Others	4	0	0.4	0	0	0	0

[a] Cases/100,000 children younger than 2 years old.
[b] Cases/100,000 children younger than 5 years old.

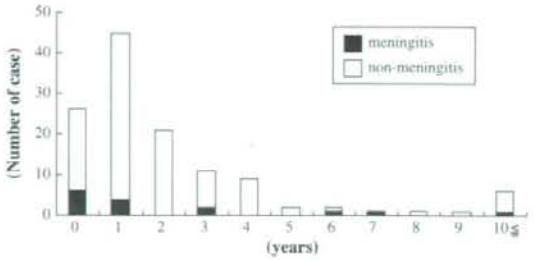

Figure 1 Age distribution of invasive pneumococcal diseases in children.

of age (19.5–23.8 and 12.6–13.8 per 100,000, respectively) was similar to those rates reported in a study conducted in Germany,[8] but was much lower than the incidence reported in the pre-pneumococcal conjugate vaccine era in the United States (166 cases/10^5 children younger than 2 years of age).[9] Several hospital-based European studies have also reported higher rates in children less than 2 years of age, including: the United Kingdom,[10] 42.1; Denmark,[11] 32.5; Finland,[12] 45.3; and Spain,[13] 59.6 cases/10^5/year. In most cases, IPD is detected by blood culture. Thus, validation of the local blood culture practices could affect the incidence rates of IPD.[14] The incidence of IPD reported in the present study was obtained from only the hospitals that have pediatric wards. If our study were to include clinics and hospitals that only have outpatient clinic, the incidence of IPD would be increased. In Japan, primary care physicians tend to easily prescribe antibiotics for febrile pediatric patients. This factor also may influence the incidence of IPD in our study.

This study is the first in the English literature to report the population-based incidence of IPD among Japanese children. One limitation of this study is that surveillance was restricted to children who lived in only 1 prefecture in Japan. Sakata previously evaluated the incidence of IPD in 8 hospitals located in 2 sub-prefectures in Hokkaido, northern part of Japan, from 1999 to 2004. He estimated that the incidence of IPD was 35.5 cases/10^5 children younger than 5 years of age.[15] It was higher than the rate reported in our study. To clarify the incidence of IPD in Japan, officially implemented active surveillance system should be established to monitor the occurrence of IPD throughout overall pediatric population in Japan.

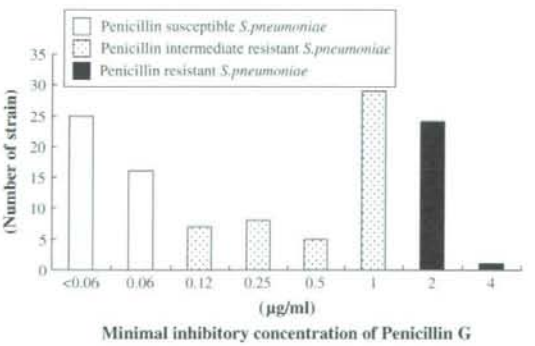

Figure 2 Antimicrobial susceptibility of isolated strains from invasive pneumococcal disease in children.

In this study, 57% of all patients of IPD cases and 63% of the pneumococcal meningitis cases involved children aged less than 2 years of age. Similar to the previous investigations, we found that almost half of the children with IPD were less than 2 years of age and that meningitis occurred more frequently in younger children than in older children.[10–12,16,17]

Our data also indicated that 21.8% of the patients with IPD have various underlying diseases and the remaining 78.2% of the patients had no underlying conditions. The most common underlying disorders in our study were congenital anomaly/syndrome, bronchial asthma and malignancy. We don't have any data on the incidence of these underlying disorders in the community. The underlying disorders in our study were different from those in other studies.[18,19] For instance, the most common underlying disorders in Bennett et al.'s and Levine et al.'s studies were sickle cell anemia and chronic pulmonary disorder, respectively. This finding supports that pneumococcal conjugate vaccine should be recommended not only for children with identifiable risk factors but children who do not exhibit any underlying disorder.

In our study, 42.6% of isolates exhibited intermediate penicillin G resistance and 21.7% of the isolates were penicillin G resistant. Although pneumococcal isolates that are resistant to antimicrobial drugs have been detected on all continents, the rate of beta-lactam resistance in Japan is one of the highest in the world.[20,21] Several reasons for the high beta-lactam resistant rate observed in Japan are considered. One reason is that oral cephalosporin antibiotics are overprescribed for outpatients as a first-choice antibiotic. The prescription of antibiotics for pediatric outpatients in Japan differs from patterns in other countries.[22,23] The problem is reflected by the observation that many *S. pneumoniae* isolates in Japan decrease their susceptibility to cephalosporin antibiotics.[24] The relatively high population density in Japan is a secondary factor, which results in easy transmission of resistant strain among children. Studies conducted in areas with a higher prevalence of penicillin-resistant *S. pneumoniae* have shown this to be associated with increased mortality.[25] In this study, pneumococcal meningitis was the most important cause of long-term morbidity, which contributed to 100% of the sequelae. The mortality rate for our study (1.6%; 2/125) was low, similar to those of other studies, i.e., 1.3%,[12] 2.0%,[26] 2.2%,[27] but not zero. There were 2 fatal cases in our study. One was fatal sepsis and the other was pneumonia. Both cases had severe underlying disease. As such, vaccination is one of the possible ways to prevent this type of infection.

At present, 7-valent conjugate pneumococcal vaccines are not yet being used in Japan. Serotyping was not performed on all of the isolated strains in each hospital in this study period and all strains were not stocked. Therefore, regrettably, serotyping of the isolated strains was not done in this study. However, another study in Japan revealed that 76.2% of the isolated strains from pneumococcal meningitis were covered by the 7-valent pneumococcal conjugate vaccine. The most frequent serotypes were 6B, 19F, 23F, 6A

and 14 in children and the same serotypes predominated among penicillin-resistant strains.[21] Serotyping of *S. pneumoniae* has to be included in the IPD surveillance system in Chiba prefecture that started in 2007.

In conclusion, the annual incidence of pediatric IPD has remained constant during the study period. Two-third of isolated strains were at least partially resistant to penicillin G. Establishment of appropriate antibiotic therapy against IPD due to penicillin-resistant strains and the introduction of pneumococcal conjugate vaccine for the prevention of IPD are the emergent issues in Japan. However, before deciding to introduce such a routine immunization program in Japanese children, nationwide surveillance for incidence of IPD, antibiotic susceptibilities and serotypes of *S. pneumoniae* isolated from IPD patients should be done.

Competing interests

The authors have no competing interests. No funding has supported this work.

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SHORT REPORT

Disseminated Bacillus Calmette-Guérin lymphadenitis in a patient with gp91*phox*$^-$ chronic granulomatous disease 25 years after vaccination

Koichi Kusuhara · Shouichi Ohga · Takayuki Hoshina · Mitsumasa Saito · Yuka Sasaki · Masataka Ishimura · Hidetoshi Takada · Masaki Fujita · Toshiro Hara

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Abstract A boy developed ipsilateral axillary lymphadenitis after Bacillus Calmette-Guérin (BCG) inoculation at the age of 5 months. Subsequently, he was diagnosed with X-linked chronic granulomatous disease (CGD) by the nitroblue tetrazolium assay when he was 4 years old. Body computerized tomography (CT) performed at the age of 25 years showed enlarged lymph nodes in the left periclavicular and axillary regions, and was confirmed by gallium scintigraphy. Mycobacterial culture, smear, and polymerase chain reaction (PCR) of the sputum and gastric fluid were negative. Whole-blood IFN-γ assay was negative as well. *Mycobacterium bovis* BCG was isolated from the lymph node biopsy by PCR amplification and culture. No mutation of the IFN-γ receptor 1 could be identified. In conclusion, CGD can be the underlying condition for BCG-itis; whole-blood IFN-γ assay might be useful in differentiating BCG infection and tuberculosis in CGD patients; BCG vaccination is contraindicated in X-linked CGD.

Keywords BCG · Chronic granulomatous disease · *Mycobacterium bovis* · Lymphadenitis · Adverse effects · Adult

K. Kusuhara · S. Ohga (✉) · T. Hoshina · M. Saito · Y. Sasaki · M. Ishimura · H. Takada · T. Hara
Department of Pediatrics, Graduate School of Medical Sciences, Kyushu University,
3-1-1 Maidashi, Higashi-ku,
Fukuoka 812-8582, Japan
e-mail: ohgas@pediatr.med.kyushu-u.ac.jp

M. Fujita
Department of Clinical Medicine,
Research Institute for Diseases of the Chest, Kyushu University,
Fukuoka, Japan

Abbreviations

CGD	Chronic granulomatous disease
BCG	Bacillus Calmette-Guérin
IFN	Interferon
IL	Interleukin
IFNGR1	IFN-γ receptor 1
PID	Primary immunodeficiency diseases
RD1	Region of difference 1
ESAT-6	Early secreted antigenic target 6-kDa protein
CFP-10	10-kDa culture filtrate protein

Chronic granulomatous disease (CGD) is an inherited disorder of phagocyte dysfunction, leading to susceptibility to pyogenic bacteria, fungi, and mycobacteria, that usually begins early in life. Although Bacillus Calmette-Guérin (BCG) vaccination is contraindicated in CGD patients for its complications, some of the patients receive it before being diagnosed and develop disseminated or regional BCG infections, known as BCG-osis and BCG-itis, respectively. These adverse effects of BCG in CGD patients usually occur in the first decade of life, especially within 1 year after vaccination [1, 4], and the late exacerbation has been rarely, if ever, reported in adults with CGD. We report a young adult with X-linked CGD, who developed disseminated lymphadenitis due to being BCG-inoculated at 5 months of age.

A 25-year-old Japanese male was admitted to our hospital because of nocturnal fever and growing supraclavicular tumors. He was diagnosed as having X-linked CGD with missense mutation of exon7 of the gp91*phox* gene (codon 257 TGC>CGC, Cys>Arg) at 4 years of age, when his elder brother was diagnosed with the same disease. The patient showed detectable but very low levels of H_2O_2-producing cells (2%), assessed by the nitroblue

tetrazolium (NBT) assay. He developed ipsilateral axillary lymphadenitis a few months after the BCG inoculation at 5 months of age. After the surgical resection of the lesion, his life has been uneventful, except for occasional eczema in adulthood. Physical examination on admission revealed non-tender enlarged lymph nodes with elastic consistency in the left cervical, supraclavicular, and axillary regions (1 cm, 2 cm, and 3 cm in diameter, respectively). Bacterial examinations on mycobacteria, including smear, culture, and polymerase chain reaction (PCR) were negative for sputum and gastric fluid. Whole-blood interferon (IFN)-γ assay (QuantiFERON-TB2G; Cellestis, Carnegie, Australia) was negative. Chest roentgenogram showed left hilar adenopathy. Body computerized tomography (CT) disclosed multiple, partly calcified, enlarged lymph nodes in the left supra- and periclavicular, left axillary, mediastinal, bilateral hilar, and paraaortic regions (Fig. 1a). Gallium scintigraphy revealed abnormal accumulation of the isotope in the lesions (Fig. 1b). The biopsy of supraclavicular lymph nodes revealed epithelioid granuloma. The specimen was positive for acid-fast bacteria. PCR amplification of the lesion using Cobas Amplicor® test (Roche Diagnostics, Grenzach-Whylen, Germany) was positive for *Mycobacterium tuberculosis* complex DNA. Culture of the specimen for 3 weeks yielded *M. bovis*, which was subsequently identified as *M. bovis* BCG substrain Tokyo by multiplex PCR [7]. Because of the spread in the thoracic lymph nodes, disseminated BCG lymphadenitis was diagnosed. After the initiation of anti-tuberculous drugs (isoniazid and streptomycin) and twice-weekly IFN-γ treatment, lymphadenitis began to be resolved (Fig. 1c). He did not tolerate rifampin. Among the defects in the interleukin (IL)-12/23-IFN-γ circuit [2], only partial dominant IFN-γ receptor 1 (IFNGR1) deficiency has been identified as a genetic basis of adverse effects of BCG vaccination in Japanese to date [6]. No mutation of the *IFNGR1* gene was found in the patient.

The mechanism of increased susceptibility to BCG in CGD patients remains to be elucidated. However, as BCG organisms are catalase-positive and are not killed by CGD phagocytes [3], it is possible that impaired oxidative burns in phagocytes may confer susceptibility to BCG in CGD patients in the absence of impaired cellular immunity or genetic defects in the IL-12/23-IFN-γ circuit [1, 4]. In the presented patient, it was established that BCG organisms had persisted intracellularly for more than 20 years and its

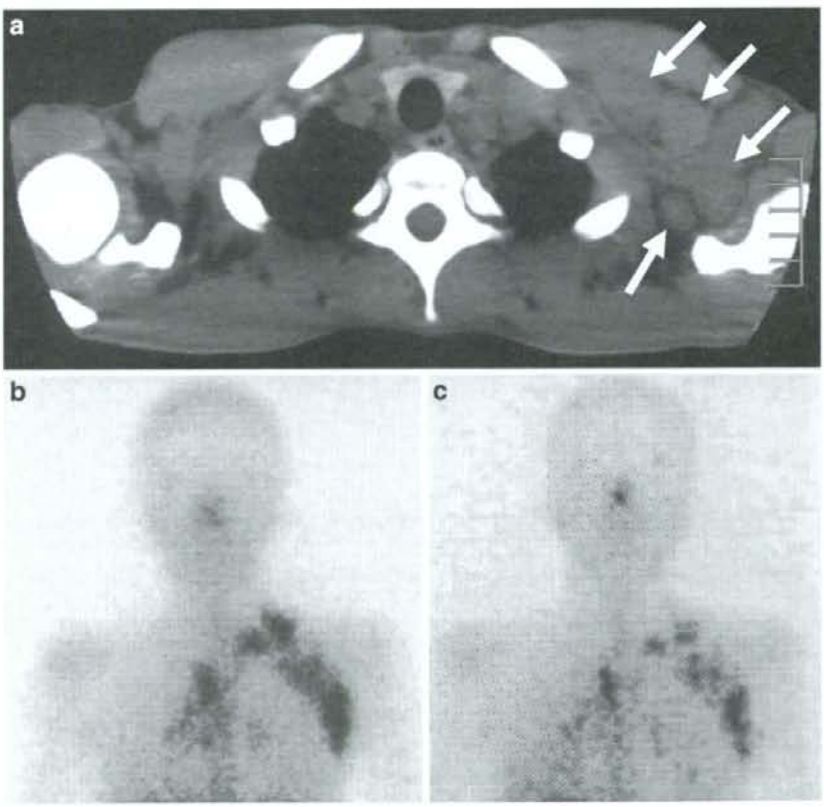

Fig. 1 Findings of the computerized tomography (CT) and gallium scintigraphic scans. **a** CT scan on admission shows enlarged lymph nodes in the left periclavicular and axillary regions (*arrows*). **b** Gallium scintigraphic scan on admission shows abnormal accumulation of the isotope in the left cervical, supra- and periclavicular, axillary, and bilateral hilar lymph nodes. **c** Gallium scintigraphic scan 2.5 months after the initiation of the treatment showed the resolution of abnormal isotope accumulation