Table 2 Parameters related to IgG subclasses, natural logarithm transformed urinary b2MG/Cr values, and admission duration

Parameters		Univariate analyses	Decision tree analyses	Multiple regression analyses	
Dependent	Independent	*p* values	Sum of squares	Beta	*p* value
IgG1					
Outpatient clinics	Age	0.0043	685239	−0.22	0.002
	Cough	0.020			
On admission	CRP	0.021	12668		
	LDH	0.0029			
	CK	0.047			
	IgG	<0.0001	400642	1.1	0.000
	Max RR	0.0032	42510		
	Max PR	0.013			
	Atelectasis (+)	0.029	195409		
	Atelectasis score	0.042			
	Inhalation Tx with beta 2 agonist by Inspiron	0.015			
IgG2					
Outpatient clinics	Age	<0.0001	122824	0.36	0.037
	Maoto Tx	0.0027			
	Duration of Maoto Tx	0.032			
On admission	LDH	<0.0001	322322		
	IgG	0.0002			
	IgA	0.0014			
	Max RR	0.029			
	Max PR	0.0008	8375		
	Gamma globulin Tx	0.028			
IgG3					
Outpatient clinics	Past history of asthma	0.008	5244	−0.39	0.008
	Past history of allergic diseases other than asthma	0.0040	519		
On admission	CK	0.023	6860		
	Inhalation Tx with beta 2 agonist by inspiron	0.0055			
IgG4					
Outpatient clinics	Age	0.033	11510		
On admission	IgG	0.017			
	CRP	0.024	8953		
	LDH	0.021	1379		
	Duration of Maoto Tx	0.048			
U-log(b2MG/Cr)					
Out patient clinics	NI Tx	0.0018	3.0		
	Duration of NI Tx	0.0014	1.8	−0.28	0.026
	Duration from the onset of Flu	0.0014			
On admission	CK	0.030			
	Max RR	<0.0001	35.0	0.32	0.019
	Max PR	0.0006	1.3		
	Duration necessary for defeverscence <37.5 °C	0.0010			
	Inhalation Tx with beta 2 agonist without inspiron	0.0014			
	Methylprednisolone IV Tx	<0.0001	5.8	0.33	0.017
	Gamma globulin Tx	0.050			

Table 2 continued

Parameters		Univariate analyses	Decision tree analyses	Multiple regression analyses	
Dependent	Independent	*p* values	Sum of squares	Beta	*p* value
Admission duration					
Outpatient clinics	Age	0.030			
	SpO_2	0.0063			
	Past history of asthma	0.0003	6.9	0.36	0.002
	Past history of allergic diseases other than asthma	0.030			
On admission	Duration after onset of FLU	0.0083			
	CRP	0.016	4.9		
	CK	0.016			
	IgG	0.011			
	Atelectasis (+)	0.0052			
	Atelectasis score	0.0017			
	Max BT	0.0006	24.7		
	Max RR	<0.0001	125		
	Max PR	0.0040	0.9		
	Inhalation Tx with beta 2 agonist by inspiron	0.015			
	Inhalation Tx with beta 2 agonist without inspiron	0.0002			
	Duration of NI Tx	0.0028	58.8	0.27	0.017
	Total XP scores	0.026	5.6		

Corrected R^2 values in decision tree analyses for IgG1, IgG2, IgG3, IgG4, u-log(b2MG/Cr) and admission duration by fivefold cross-validation methods are 0.64, 0.55, 0.25, 0.26, 0.42, and 0.52, respectively. *p* values of ANOVA for the above parameters are from 0.000 to 0.003 except IgG4 (0.067). Corrected R^2 values in ANOVA for IgG1, IgG2, IgG3, IgG4, u-log(b2MG/Cr), and admission duration are 0.94, 0.36, 0.22, 0.057, 0.44, and 0.55, respectively. Only the data for *p* values $\leq$0.05 are shown

U-log(b2MG/Cr) natural logarithm transformed urinary b2MG/creatinine, *NI* neuraminidase inhibitors, *Inspiron* a device for a continuous nebulized formulation, *BT* body temperature, *PR* pulse rate, *RR* respiration rate, *Max* maximum, *Tx* therapy, *IV* intravenous administration

Table 3 Significant parameters for IgG subclasses, natural logarithm transformed urinary b2MG/Cr values, and admission duration by multiple regression analyses

Dependent parameters	Independent parameters	Multiple regression analyses	
		Beta	*p* value
IgG1	IgG2	0.49	0.000
	IgG3	0.35	0.006
IgG2	IgG1	0.62	0.000
	IgG3	−0.29	0.037
IgG3	IgG1	0.52	0.004
	IgG2	−0.36	0.037
IgG4	IgG1	0.41	0.007
u-log(b2MG/Cr)	IgG1	−0.32	0.035
Admission duration	IgG3	−0.37	0.015

p values of ANOVA for IgG1, IgG2, IgG3, IgG4, natural logarithm transformed urinary b2MG/creatinine(u-log(b2MG/Cr)) values, and admission duration are 0.000, 0.000, 0.012, 0.007, 0.035, and 0.015, respectively. R^2 values are 0.42, 0.32, 0.16, 0.15, 0.082, and 0.11, respectively. Only the data for *p* values <0.05 are shown

times per minute (range, 36–44), 39.1 °C (range, 38.7–39.4 °C), and 142 times per minute (range, 135–149), respectively. The duration necessary for defeverscence below 37.5 °C for the first time ($n = 45$) was 19.2 h (range, 14.8–23.6). Neuraminidase inhibitors ($n = 45$) and maoto therapy ($n = 45$) were scheduled to be given for 5 days; however, the doses were for 6.2 days (range, 5.5–6.9) and 5.2 days (range, 4.8–5.5), respectively, depending on the medical condition of the patients.

Serum levels of IgG, IgM, and IgA ($n = 24$) were 945 mg/dl (range, 840–1,051), 119 mg/dl (range, 104–134), and 119 mg/dl (range, 90–147), respectively. Serum concentrations of IgG subclasses ($n = 45$) were as follows: IgG1, 653 mg/dl (594–712); IgG2, 252 mg/dl (214–289); IgG3, 43.7 mg/dl (36–51); and IgG4, 28 mg/dl (18–39). All these values were within normal range compared to age-matched normal Japanese controls [17]. Blood cell counts indicated WBC count ($n = 45$), 8,598/μl (7,325–9,871); percentage of granulocytes ($n = 36$), 71 % (64–77 %); normal levels of platelets ($n = 45$), 24.5 × 10^4/μl (22.2–26.7). Fibrinogen degradation product (FDP)

Table 4 Correlations between clinical parameters

		rs	*p* value
1.	Age		
	Maximal respiration rate	−0.50	0.0004
	IgG	0.61	0.0014
2.	Maximal respiration rate		
	IgG	−0.58	0.0029
	Intravenous steroid IV therapy on admission	0.49	0.0006
	Duration of NI Tx in outpatient clinics	−0.30	0.049
3.	IgG		
	Intravenous steroid IV therapy on admission	−0.43	0.036
4.	Past history of asthma		
	Intravenous steroid IV therapy on admission	0.32	0.030
5.	Intravenous steroid therapy on admission		
	Duration of NI Tx in outpatient clinics	−0.33	0.026
6.	Duration of NI Tx in outpatient clinics		
	Duration of NI Tx on admission	0.35	0.019

rs denotes *r* values by Spearman's correlation analysis. Only the data for *p* values <0.05 are shown

NI neuraminidase inhibitors, *Tx* therapy, *IV* intravenous administration

(D-dimer) levels ($n = 18$) were 1.6 μg/dl (0.95–2.2). Biochemical markers revealed CRP ($n = 45$), 2.0 mg/dl (1.3–2.7); lactate dehydrogenase (LDH) ($n = 43$); 264 IU/l (244–283); and creatinine kinase (CK) levels ($n = 43$), 145 IU/l (112–178). In contrast, natural logarithm transformed urinary b2MG/creatinine values ($n = 43$) were elevated at 7.5 μg/g Cr (7.1–8.0).

The incidence of atelectasis and pneumonia were 22 % (10/35) and 87 % (39/45), respectively. Chest roentgenogram severity was evaluated by summing the number of atelectasis in the bilateral lungs (atelectasis score), 0.3 (0.1–0.4), and the pneumonia score, which was based on the area of pneumonia: 0 for no pneumonia, 1 and 2 for pneumonia of which the area was under or over half of the unilateral lung, respectively, 2.4 (2.0–2.8). A total chest roentgenogram score (chest Xp score), which was calculated by summing both the atelectasis and pneumonia scores, was 2.7 (2.2–3.2).

Results for H1N1 pdm09 surveillance in the study period (Table 1)

After July 24, 2009, all the cases were not necessary to be confirmed by PCR for the diagnosis of H1N1 pdm09 in Japan. Then, according to pathogen surveillance, specimens obtained in the pathogen sentinels and those obtained from patients with serious pneumonia, encephalopathy, or who had died were sent to the prefectural and municipal public health institutes for virus isolation and PCR diagnosis in the three public health institutes in Osaka Prefecture, including Osaka Prefectural Institute of Public Health. Almost all cases of influenza A were diagnosed as H1N1 pdm09.

Parameters related to IgG subclasses, natural logarithm transformed urinary b2MG/Cr values, and admission duration (Table 2)

A past history of asthma was significantly associated with serum IgG3 levels ($p = 0.008$). The duration of neuraminidase inhibitors for outpatient clinics ($p = 0.026$), maximal respiration rate from vital signs ($p = 0.019$), and intravenous administration of methylprednisolone on admission ($p = 0.017$) were statistically significant parameters for natural logarithm transformed urinary b2MG/Cr values. The presence of a past history of asthma at outpatient clinics ($p = 0.002$) and duration of neuraminidase inhibitor therapy on admission ($p = 0.017$) significantly influenced on admission duration.

Significant parameters for IgG subclasses, natural logarithm transformed urinary b2MG/Cr values, and admission duration by multiple regression analyses (Table 3)

Natural logarithm transformed urinary b2MG/Cr and admission duration were significantly influenced by IgG1 and IgG3, respectively.

A mean covariance structure equation model

Path analysis was performed by using a mean covariance structure analysis. However, the model did not achieve a satisfactory fit: CFI = 0.94, RMSEA = 0.098, AIC = 184. Therefore, we constructed another model in the absence of IgG4 because we could find no useful parameters associated with IgG4. This path analysis revealed a better fit: CFI = 1.000, RMSEA = 0.000, AIC = 148. Finally, we tried to improve the model by deleting the partial correlations between the two corresponding parameters with a *p* value of more than 0.05. The final model (Fig. 1) achieved the most satisfactory fit: CFI = 1.000, RMSEA = 0.000, AIC = 145.

In the path analysis by a mean covariance structure analysis, we are able to evaluate three kinds of effects between the two parameters: direct (for example, from A to C), indirect (for example, from A to C via B), and total effects, which are summing up of the direct and indirect effects. In clinical medicine, total effects seem to be important because they include comprehensive effects.

Direct effects of path analysis (Table 5) revealed that the estimated clinical severity was significantly associated with a short duration of neuraminidase therapy in

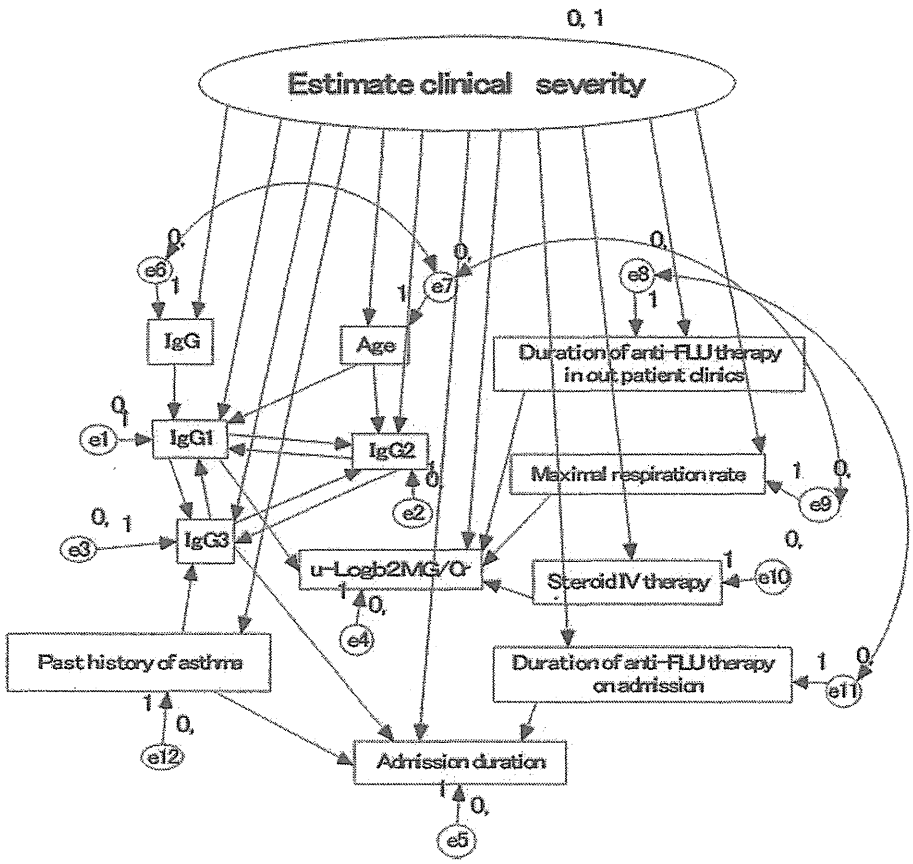

Fig. 1 Path analysis for the estimated clinical severity of pH1N1 pdm09 influenza. The covariance of estimated clinical severity was fixed as 1. The stable index was 0.15. The final model achieved the most satisfactory fit: CFI = 1.000, RMSEA = 0.000, R^2 value for admission duration = 0.67, AIC = 145. *Anti-FLU therapy* neuraminidase inhibitor therapy, *Steroid* methylprednisolone, *IV* intravenous administration, *u-log(b2MG/Cr)* natural logarithm transformed urinary beta-2MG/creatinine values, *b2 agonist* beta 2 agonist

Table 5 Direct effects of path analysis

Origin	End	Standardized path coefficient	*p* value
Estimated clinical severity	Max RR	0.75	<0.001
Estimated clinical severity	Admission duration	0.55	<0.001
Estimated clinical severity	Steroid IV Tx	0.53	<0.001
Estimated clinical severity	Past history of asthma	0.43	0.011
Estimated clinical severity	IgG	−0.43	0.009
Estimated clinical severity	Duration of NI Tx in outpatient clinics	−0.39	0.017
Duration of NI Tx in outpatient clinics	u-log(b2MG/Cr)	−0.36	0.002
IgG1	u-log(b2MG/Cr)	−0.27	0.024
Age	IgG2	0.49	<0.001
Duration of NI Tx on admission	Admission duration	0.33	0.002

Only the data for *p* values <0.0241 are shown

NI neuraminidase inhibitors, *Tx* therapy, *IV* intravenous administration, *u-log(b2MG/Cr)* natural logarithm transformed urinary b2MG/creatinine values

outpatient clinics ($p = 0.017$). Moreover, duration of neuraminidase therapy in outpatient clinics and serum IgG1 significantly reduced the logarithmically transformed urinary b2MG/Cr levels, with *p* values of 0.002 and 0.024, respectively. For hospital stay, the duration of oseltamivir given on admission ($p = 0.002$) was shown to be an adverse factor.

Total path analyses using a Bayesian method (Table 6) revealed that the estimated clinical severity caused by pH1N1 pdm09 was positively associated with the logarithmically transformed urinary b2MG/Cr levels, possible evidence of elevated serum cytokine levels [14], with *p* values of <0.001, while negatively associated with the serum IgG, IgG1, IgG2, and IgG3 levels, duration of

Table 6 Total path analyses using a Bayesian method

End	Standardized path coefficient		
	Mean	SEM	*p* value
Max RR	0.68	0.01	<0.001
Admission duration	0.68	0.01	<0.001
Steroid IV Tx	0.57	0.01	<0.001
Past history of asthma	0.52	0.01	<0.001
Urinary log (b2MG/Cr)	0.46	0.01	<0.001
Duration of NI Tx on admission	0.22	0.01	<0.001
IgG3	−0.48	0.02	<0.001
IgG	−0.40	0.01	<0.001
Duration of NI Tx in outpatient clinics	−0.38	0.01	<0.001
IgG1	−0.37	0.01	<0.001
Age	−0.26	0.02	<0.001
IgG2	−0.19	0.02	<0.001

SE standard error, *Max* maximal, *RR* respiration rate

IV intravenous administration, *Tx* therapy, *NI* neuraminidase inhibitor

neuraminidase inhibitor therapy in outpatient clinics, and age with p values of <0.001, respectively.

Discussion

Pneumonia is known to be an important complication of H1N1 pdm09 [1]. Several risk factors were reported to adversely affect influenza infection, but it was shown that healthy people also suffered from pneumonia caused by the pandemic influenza virus [3]. Thus, the question remains why only a certain percentage of people suffered from pneumonia. It was speculated from the experiments using ferrets that this was caused by the difference between the amount of viral load and immunological defense against viral infection in patients [4]. Also, recent investigations suggested additional possibilities of an association of oxygen stress caused by the influenza viral infection [18] and a state of vitamin D deficiency [19] in children.

There are few investigations regarding the role of IgG subclasses in H1N1 pdm09 infection. In this article, we offer the first evidence that serum IgG and its subclasses were able to reduce the estimated clinical severity by H1N1 pdm09 infection as calculated by path analyses. These observations were supported by a recent report by Gordon et al. [20], which suggested the effectiveness of pooled human immunoglobulin for critically ill patients with pneumonitis caused by influenza H1N1 pdm09. Immunoglobulin therapy was proved to be useful in Spanish influenza pneumonia [21]. Also, it was speculated that serum IgG protected patients against pneumonia caused by seasonal influenza infection; this was supported by the evidence that IgG was a dominant antibody in protection of the murine lung against seasonal H1N1 influenza [6] and that IgG was also of primary importance in the lower respiratory tract in humans [22].

Two possibilities for the effect of IgG and its subclasses were considered in this article. One was the presence of a neutralizing antibody to protect against H1N1 pdm09 before the onset of influenza infection and the other was a newly synthesized antibody caused by the present influenza infection.

Our data were compatible with the latter hypothesis because the neutralizing antibody that was clinically effective to protect against H1N1 pdm09 infection was rare in children [23]. Moreover, a recent report [24] revealed that a neutralizing antibody to protect against H1N1 pdm09 infection increased as early as 10 days after vaccination against H1N1 pdm09. In addition, a stronger response to increase the neutralizing antibody to H1N1 pdm09 was observed in individuals with an elevated preexisting seasonal influenza antibody. Recently, these data were confirmed by another report [25] in which the author showed only 7 days were necessary for an adequate increase in neutralizing antibody to protect against H1N1 pdm09 infection. Here, we measured serum levels of IgG and its subclasses from 5 to 10 days after the onset of pH1N1 pdm09 infection, which was estimated from data on the incubation period of H1N1 pdm09 (1–3 days), duration periods before admission (2–3 days), and blood tests to measure serum IgG and its subclasses levels (2–3 days) performed after admission. Thus, the effect of serum IgG and its subclasses on reducing the estimated clinical damage caused by the pH1N1 pdm09 infection in our cases was possibly derived from the newly synthesized neutralizing antibody. Although two patients received vaccinations for H1N1 pdm09 once before admissions, we could find no significant differences in the serum levels of IgG subclasses between the two and other nonvaccinated patients (data not shown). This finding was speculated to derive from the small number of the patients. Also, neither had clinical histories of influenza-like signs and symptoms before admissions.

For IgG2, Gordon et al. [6] reported low serum levels of IgG2 in severe pneumonia patients caused by H1N1 pdm09. Recently, this observation was supported by Chan et al. [7], and the authors also suggested that this was not derived from a genetic abnormality, but increased cytokine levels. In our study, we found no significant effect of serum IgG2 on the estimated clinical severity caused by pH1N1 pdm09 in the direct path analyses (Table 5), although it worked significantly against estimated clinical severity in the total path analyses (Table 6). Because the results of total path analyses included both direct and indirect effects, they were thought to represent the comprehensive effects

of IgG2 in this study. Thus, it was possible that the foregoing data were related to the protective role of IgG2 in bacterial coinfection in H1N1 pdm09 because IgG2 is known to antagonize pneumococcal and *Haemophilus influenzae* type b antigens [26]. In fact, coinfection with these bacteria were reported in H1NI pdm09 infection [27, 28]. Also, *Haemophilus influenzae* was recently reported to accelerate the severity of seasonal influenza A (H1N1) PR8 virus infection using a mouse model [29].

There are no reports discussing the role of serum levels of IgG3 in H1N1 pdm09 infection. In this article, we showed that the estimated clinical severity caused by pH1N1 pdm09 was associated negatively with serum IgG3 levels by the total path analysis ($p < 0.001$). IgG3 was speculated to eliminate the infected cells with pH1N1 pdm09 through antibody-dependent cellular toxicity (ADCC) and complement-dependent cellular cytotoxicity (CDC) with its highly flexible hinge, which easily recognizes antigens and has the best binding affinity to C1q for a greater ability to trigger effector functions [30]. The complement C1q was reported to enhance the biological activity for hemagglutinin inhibition activity of the seasonal influenza A (H1N1) PR8 virus in the presence of IgG3 in an experiment on SCID mice [31]. Also, IgG3-associated immune complexes were reported to bind human neutrophils, cells that mediate ADCC, three times faster than those of IgG1 [32]. In fact, we experienced a patient with IgG3 deficiency [13] who needed a significantly longer duration of admission and also had elevated markers for inflammation such as CRP, CK, and urinary excretion of b2MG/Cr, an index of elevated serum cytokine levels [14].

Serum levels of IgG4 were reported to increase age dependently [17]. Because we found neither abnormality nor statistically significant parameters related to serum IgG4 levels in our pH1N1 pdm09 patients, we excluded the IgG4 data to achieve a better fit model in the mean covariance structure equation analysis.

In this article, we used mean covariance structure equation analysis to clarify the association of clinical severity of pH1N1 pdm09 with several clinical parameters. This analysis has recently proposed as a powerful analysis equal or superior to the conventional linear regression analysis [11]. In particular, this method enabled us to investigate the statistical model including nonmeasured parameters (latent variables) [10, 11]. In our case, we regarded the latent variable as the estimated clinical severity in patients with pH1N1 pdm09. Our hypothesis was confirmed by evidence that the estimated clinical severity showed significantly positive association in the direct path analysis with maximal respiration rate, duration of admission, past history of asthma, and steroid infusion therapy. The former two factors were clinically reasonable and the latter two were supported by the literature, respectively [3, 33].

The question might arise why we were able to show that admission duration was a significant marker for the clinical severity of pH1N1 pdm09 infections. Because the discharge criteria seem to be similar to the other hospitals, the reason we found a difference in admission duration was speculated to derive from increased statistical power by using the mean covariance structure equation model. In fact, R^2 value for admission duration in our study was 0.67.

There are several limitations to this report. First, we could investigate serum levels of IgG subclasses for only 45 patients of pH1N1 pdm09. Also, we had no data for IgG subclasses on patients with milder symptoms and healthy volunteers. These limitations were caused by a financial problem. Second, we could not measure a time-dependent neutralizing antibody titer to influenza H1N1 pdm09. Third, not all the patients in our study were confirmed as H1N1 pdm09 by RT-PCR. Fourth, we could not show laboratory evidence that IgG3 was involved in the ADCC or CDC responses to influenza pH1N1 pdm09.

In conclusion, we were able to show the theoretical role of serum IgG and its subclasses in pH1N1 pdm09 infection by using a mean covariance structure equation model for the first time. The results of this analysis led us to propose a protective role of IgG and its subclasses in pH1N1 pdm09 infection. Further study is necessary to elucidate the mechanism of IgG and its subclasses in protecting against influenza pH1N1 pdm09 infection in children.

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Conflict of interest The authors have declared that no competing interests existed.

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Phase II and III Clinical Studies of Diphtheria-Tetanus-Acellular Pertussis Vaccine Containing Inactivated Polio Vaccine Derived from Sabin Strains (DTaP-sIPV)

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Background. Phase II and III clinical studies were conducted to evaluate immunogenicity and safety of a novel DTaP-IPV vaccine consisting of Sabin inactivated poliovirus vaccine (sIPV) and diphtheria-tetanus-acellular pertussis vaccine (DTaP).

Methods. A Phase II study was conducted in 104 healthy infants using Formulation H of the DTaP-sIPV vaccine containing high-dose sIPV (3, 100, and 100 D-antigen units for types 1, 2, and 3, respectively), and Formulations M and L, containing half and one-fourth of the sIPV in Formulation H, respectively. Each formulation was administered 3 times for primary immunization and once for booster immunization. A Phase III study was conducted in 342 healthy infants who received either Formulation M + oral polio vaccine (OPV) placebo or DTaP + OPV. The OPV or OPV placebo was orally administered twice between primary and booster immunizations.

Results. Formulation M was selected as the optimum dose. In the Phase III study, the seropositive rate was 100% for all Sabin strains after primary immunization, and the neutralizing antibody titer after booster immunization was higher than in the control group (DTaP + OPV). All adverse reactions were clinically acceptable.

Conclusions. DTaP-sIPV was shown to be a safe and immunogenic vaccine.

Clinical Trials Registration. JapicCTI-121902 for Phase II study, JapicCTI-101075 for Phase III study (http://www.clinicaltrials.jp/user/cte_main.jsp).

Keywords. clinical study; combination vaccine; DTaP; IPV; Sabin strain.

Since the World Health Organization (WHO) launched the global polio eradication program in 1988, the number of patients with polio caused by wild-type polioviruses worldwide has steadily decreased from a 1988 estimate of approximately 350 000 cases to 67 cases on 5 June 2012 [1]. The oral polio vaccine (OPV) has played a leading role in the program as not only is it very effective and safe but it also has several important advantages, including low cost, ease of administration, and induction of superior mucosal immunity, compared to inactivated polio vaccine (IPV). In polio-free areas, however, concerns have been raised regarding 2 negative aspects of OPV as a live vaccine: One issue is a risk of causing vaccine-associated paralytic poliomyelitis (VAPP) in OPV vaccinees and those in contact with them, although this occurred very infrequently [2]. The other issue is a risk of polio epidemics caused by vaccine-derived polioviruses (VDPVs), which have highly neurovirulent and transmissible properties [3]. As long as OPV is in use, it will be impossible to avoid the occurrence of VAPP and polio caused by VDPVs. Therefore, many countries in which polio has been eradicated have been shifting from OPV to IPV.

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In addition to conventional IPV produced using virulent polioviruses (cIPV), a novel IPV produced using nonvirulent poliovirus seed strains, such as the attenuated Sabin strains, is now under investigation. However, with a view to eradicating wild-type polioviruses, regulations have been placed on manufacturing of bulk cIPV from virulent poliovirus strains at new manufacturing sites [4]. On the other hand, manufacturing of an IPV using attenuated Sabin strains (sIPV) is not regulated because it is made from attenuated Sabin strains. Moreover, the use of attenuated Sabin strains not only provides additional safety during vaccine production, but also reduces the risk of recurrent polio epidemics in the unlikely event of an unintentional or intentional release into the human population or environment. Therefore, manufacturing of sIPV is also encouraged by the WHO in consideration of social safety and IPV supply post polio eradication [5, 6].

The first Phase I clinical study using an sIPV was carried out in the United States starting in 1985 to evaluate its safety and immunogenicity [7]. Since then, many institutes have been continuing the development of sIPV to further optimize doses through preclinical and clinical studies [8–10].

The Chemo-Sero-Therapeutic Research Institute (Kaketsuken) initiated development of a diphtheria-tetanus-acellular pertussis (DTaP) vaccine that contains a domestically produced sIPV (DTaP-sIPV). The optimum dose of the sIPV in DTaP-sIPV was determined in a Phase II clinical study, and the safety and immunogenicity of DTaP-sIPV manufactured at the optimum dose of the sIPV were evaluated in a Phase III clinical study. This is the first report on the immunogenicity and safety of the sIPV in the Phase III study.

MATERIALS AND METHODS

Vaccines

Type 1, type 2, and type 3 sIPV bulk stocks manufactured by Japan Poliomyelitis Research Institute were purchased to manufacture DTaP-sIPV [10]. The following Sabin strains were used to manufacture the sIPV bulks: LSc, 2ab strain for type 1; P712, Ch, 2ab strain for type 2; and Leon, $12a_1b$ strain for type 3.

Given a previous report that the immunogenicity of sIPV differs from that of cIPV, it was necessary to select the optimum dose of sIPV [11]. The National Institute of Infectious Diseases, Japan, played a central role to determine the required D-antigen units (DU) content of sIPV that would be equivalent to the WHO reference IPV (91/574) or cIPV derived from virulent polioviruses (type 1 : type 2 : type 3 = 40 DU : 8 DU : 32 DU) in terms of immunogenicity using rats, and concluded that the relevant DU of sIPV is 3, 100, and 100 DU for types 1, 2, and 3, respectively [10]. Guoyang Liao et al [9] conducted a Phase II clinical study of sIPV in China, and reported that the appropriate concentrations of sIPV were 15 DU, 32 DU, and 45 DU for types 1, 2, and 3, respectively. However, regarding the methods of testing for D-antigen content, the antibody and standards that Guoyang Liao et al used in their study differed from those used in the present study. Therefore, we could not compare the antigen contents. As the DTaP components, antigens (diphtheria toxoid, tetanus toxoid, pertussis toxoid [PT]) and filamentous hemagglutinin (FHA) manufactured by Kaketsuken were used [12–16].

The final bulk of DTaP-sIPV was prepared by mixing a trivalent sIPV with the licensed DTaP final bulk. Syringes were used as single-dose containers of the final product. In addition to Formulation H, a DTaP-sIPV containing the same amount as the sIPV (type 1 : type 2 : type 3 = 3 DU : 100 DU : 100 DU), Formulations M and L, containing half and one-fourth of the amount in the sIPV, respectively, were prepared to determine the optimum dose of sIPV in DTaP-sIPV in the Phase II study. These formulations differed only in the amount of sIPV. In the Phase III study, the DTaP vaccine (manufactured by Kaketsuken), the OPV vaccine (Japan Poliomyelitis Research Institute), and an OPV placebo (Japan Poliomyelitis Research Institute), excluding the active ingredient (Sabin viruses) from the OPV vaccine, were used as control drugs. The DTaP vaccine contained 4 U (pertussis protective unit) or more of *Bordetella pertussis* protective antigen, 16.7 limit of flocculation (Lf) or less of diphtheria toxoid, and 6.7 Lf or less of tetanus toxoid per dose. The OPV contained 6.0, 5.0, and 5.5 $\log_{10}$ 50% cell culture infectious doses for Sabin types 1, 2, and 3, respectively.

Study Design

Phase II Study

The immunogenicity and safety of Formulations H, M, and L were evaluated in 104 healthy infants (age range, 3–89 months) who were recruited between 12 May and 20 June 2009. This was a multicenter, randomized, double-blind, parallel-group study. After informed consent was obtained from parents, eligible participants were randomly assigned to 3 groups (Group H, Group M, and Group L), which received Formulation H, M, or L, respectively. Formulation H, M, or L was subcutaneously administered at a dose of 0.5 mL four times as follows: a series of 3 doses given at intervals of 3–8 weeks for primary immunization, and 1 dose for booster immunization 6–18 months after the third vaccination.

Phase III Study

The immunogenicity and safety of DTaP-sIPV (Formulation M) was evaluated in 342 healthy infants (age range, 3–89 months) who were recruited between 9 March and 30 June 2010, using DTaP plus OPV as a control. This was a multicenter, randomized, double-blind, parallel-group study. After informed consent was obtained, eligible participants were randomly assigned to 2 groups, Group M and the Control group, which received either Formulation M and OPV placebo, or DTaP and OPV, respectively. Formulation M or DTaP was

subcutaneously administered at a dose of 0.5 mL four times as follows: a series of 3 doses given at intervals of 3–8 weeks for primary immunization, and 1 dose for booster immunization 6–18 months after the third vaccination. OPV placebo or OPV at a dose of 0.05 mL was orally administered twice at an interval of at least 6 weeks during the period from completion of blood collection for primary immunization with Formulation M or DTaP to 35 days before booster immunization.

In the Phase II and Phase III studies, blood was collected before immunization, 4–6 weeks after primary immunization, before booster immunization, and 4–6 weeks after booster immunization to determine the antibody titers against attenuated poliovirus strains (Sabin 1, 2, and 3 strains), PT, FHA, diphtheria toxin, and tetanus toxin. The neutralizing antibody titers against virulent poliovirus strains were determined only in the Phase II study. Participants were monitored for safety by means of a health diary for 27 days after immunization. Those who received OPV placebo or OPV in the Phase III study were monitored for safety by means of a health diary for 34 days after immunization. For severity classification, the criteria were defined in Grades 1 to 4, with Grade 4 being the most severe grade. These clinical studies were conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, Good Clinical Practice, and relevant regulatory laws.

Serological Methods

The neutralizing antibody titers against polioviruses were determined by neutralization test [17]. Challenge viruses were Sabin strains (types 1, 2, and 3) and virulent poliovirus strains (type 1: Mahoney strain; type 2: MEF-1 strain; type 3: Saukett strain). The protective level of neutralizing antibody titer against Sabin strains and virulent poliovirus strains was thought to be 1:8 or more [18]. The antibody titers against PT and FHA were determined using a Wako reagent kit for anti-*Bordetella pertussis* antibody titers. The protective level of antibody titers against both PT and FHA was 10 enzyme-linked immunosorbent assay (ELISA) units (EU)/mL [19]. The neutralizing antibody titer against diphtheria toxin was determined by neutralization test using Vero cells as indicator cells. The protective level of antibody titer against diphtheria toxin was 0.1 international units (IU)/mL [20]. The antibody titer against tetanus toxin was determined using a KPA kit (manufactured by Kaketsuken). In this system, the tetanus antibody titer is measured based on indirect agglutination using sensitized particles (artificial synthetic particles bound to tetanus toxoid). The protective level of antibody titer against tetanus toxin was 0.01 IU/mL [21].

Statistics

Phase II Study

For each group, the seropositive rate, the geometric mean antibody titer (GMT), and their 2-sided 95% confidence intervals (CIs) after primary and booster immunizations were calculated for all active components. In terms of sample size, from the results of an immunogenicity examination of the primary immunization conducted in the previous clinical trial, as well as immunogenicity tests in *Macaca fascicularis*, it was thought possible to confirm the dose response ($\alpha = .025$ [1-tailed test]; $\beta = .20$, power of 0.80, contrast method) with 20 participants or less per group. However, considering the inevitable attrition of study participants, we required 30 participants per group.

Phase III Study

The primary endpoint in this study was the seropositive rates of Group M, which were to be higher than 90% for Sabin types 1, 2, and 3 after primary immunization. The secondary endpoint was that the seropositive rates for all components of DTaP after primary immunization in Group M were not to be inferior to those in the Control group. To verify that the seropositive rates (p_1) for neutralizing antibodies against Sabin types 1, 2, and 3 in Group M were higher than the statistical tolerance limit (p_0) (90%) for the analysis of the primary endpoint, the following were tested by 1-sample binomial test with a 1-sided significance level of 2.5%: the null hypothesis that the respective seropositive rates (p_1) were equal to or lower than the statistical tolerance limit (p_0) (H_0; $p_0 \geq p_1$); and an alternative hypothesis that the respective seropositive rates (p_1) were higher than the statistical tolerance limit (p_0) (H_1; $p_0 < p_1$). To verify that the seropositive rate against PT, FHA, diphtheria toxin, or tetanus toxin in Group M was not inferior to the Control group by more than 10% for the analysis of the secondary endpoint (clinically acceptable maximum difference [s_0]), a 1-sided Farrington–Manning test was used with a significance level of 2.5%. The full-analysis set (FAS) was used to represent the immunogenicity population for the primary endpoint, because this analysis was intended to verify the superiority. The per-protocol set (PPS) was used to represent the immunogenicity population for the secondary endpoint, because this analysis was intended to verify the noninferiority. In terms of sample size, an accurate sample size of 180 participants in Group M was needed to verify that the seropositive rates (p_1) for neutralizing antibodies against Sabin types 1, 2, and 3 of the primary endpoint were higher than the statistical tolerance limit (p_0) (90%), ($\alpha = .025$ [1-tailed test]; $\beta = 0.115$, power of 0.96^3, expectation value = 97%). Because of an inevitable attrition of study participants, we required 210 patients in Group M. Eligible participants were randomly assigned in a 2:1 ratio to Group M and the Control group, respectively. Even for calculations regarding the secondary endpoint, the number of participants was sufficient.

RESULTS

Phase II Clinical Study

All 104 participants were included in the safety population, and 102 participants (Group H: 32; Group M: 38; Group L: 32) who

received the booster immunization were included in the FAS immunogenicity population (2 participants did not receive the booster immunization: 1 in Group H who withdrew consent, and 1 in Group L who moved elsewhere).

The seropositive rate was 100% for all poliovirus strains in all groups, except for virulent type 1 after primary immunization. There was no clear dose response effect among the Sabin strain groups. After primary immunization, the seropositive rates against the virulent type 1 in Groups H, M, and L were 90.9% (30/33 participants), 97.4% (37/38), and 97.0% (32/33), respectively. However, after the booster immunization, the seropositive rate was 100% for all virulent types as well as for all Sabin strains in all groups. The GMTs (log_2) against polioviruses after primary and booster immunizations are shown in Table 1. After primary and booster immunizations, no clear dose response was observed in the neutralizing antibody titers.

As for adverse reactions, the incidence of erythema at the injection site was 87.9% (29/33; 95% CI, 71.8–96.6), 73.7% (28/38; 95% CI, 56.9–86.6), and 72.7% (24/33; 95% CI, 54.5–86.7) in Groups H, M, and L, respectively. The incidence of pyrexia was 63.6% (21/33; (95% CI, 45.1–79.6), 47.4% (18/38; 95% CI, 31.0–64.2), and 48.5% (16/33; 95% CI, 30.8–66.5) in Groups H, M, and L, respectively. The incidence of adverse reactions was higher in Group H compared with Groups M and L; however, there were no statistically significant differences among the 3 groups. Considering the GMT (Table 1) and adverse reactions, we selected Formulation M containing type 1 : type 2 : type 3 = 1.5 DU : 50 DU : 50 DU of sIPV in the DTaP-sIPV as the optimum dose.

Phase III Clinical Study

In this study, the immunogenicity and safety of Formulation M, selected as the optimum dose in the previous Phase II study, were evaluated. Of 342 participants enrolled in the study, 221 and 121 participants were randomly assigned to Group M and the Control group, respectively (Figure 1). A total of 337 participants completed this study. All participants were included in the safety population. One participant in the Control group withdrew from this study after the first immunization because of relocation, with no postimmunization. Therefore, this participant was excluded from the FAS immunogenicity population. There were 336 participants in the PPS immunogenicity population, after the exclusion of protocol deviations. The baseline attributes of participants are shown in Table 2. In both groups, the preimmunization seropositive rate was high for tetanus and low for poliovirus type 3. No statistically significant differences were observed in the baseline factors between the 2 groups.

Immunogenicity

Primary Immunization

The seropositive rates and GMTs after primary immunization are shown in Table 3. The seropositive rate was 100.0% for Sabin types 1, 2, and 3 after primary immunization. The null hypothesis (seropositive rate ≤90%) was rejected by binomial test ($P < .001$), verifying that the seropositive rates of Group M were higher than 90% for Sabin types 1, 2, and 3 after primary immunization, which met the primary endpoint.

In addition, results of the Farrington–Manning test verified that Group M was not inferior to the Control group in terms of

Table 1. Poliovirus Type-Specific Neutralizing Antibody Geometric Mean Titers After Primary and Booster Immunization in Phase II Study

			GMT[a] (95% CI)		
Time Point	Poliovirus		Group H (n = 33)[b]	Group M (n = 38)[b]	Group L (n = 33)[b]
Primary immunization	Sabin strain	Type 1	9.94 (9.25–10.62)	10.26 (9.73–10.80)	10.55 (9.89–11.20)
		Type 2	10.33 (9.88–10.79)	9.79 (9.35–10.22)	9.71 (9.20–10.23)
		Type 3	9.97 (9.47–10.47)	9.93 (9.46–10.41)	9.14 (8.50–9.78)
	Virulent strain	Type 1	5.71 (4.97–6.45)	5.93 (5.50–6.37)	5.94 (5.29–6.58)
		Type 2	9.77 (9.23–10.31)	9.16 (8.68–9.63)	9.11 (8.48–9.73)
		Type 3	9.82 (9.37–10.27)	9.54 (9.07–10.01)	8.92 (8.33–9.52)
Booster immunization	Sabin strain	Type 1	12.05 (11.42–12.68)	12.50 (11.89–13.11)	12.39 (11.77–13.01)
		Type 2	13.64 (13.14–14.14)	13.79 (13.27–14.31)	12.56 (11.93–13.20)
		Type 3	12.55 (12.00–13.10)	12.75 (12.26–13.24)	11.64 (10.97–12.31)
	Virulent strain	Type 1	8.66 (8.07–9.25)	8.33 (7.70–8.96)	8.09 (7.34–8.85)
		Type 2	13.56 (12.99–14.14)	13.46 (12.86–14.06)	12.36 (11.61–13.11)
		Type 3	12.41 (11.75–13.06)	12.50 (12.02–12.98)	11.42 (10.78–12.06)

Group H received Formulation H, Group M received Formulation M, and Group L received Formulation L. Primary immunization consisted of 3 immunizations; booster immunization was the fourth immunization. Seropositive is ≥1:8(2^3).

Abbreviations: CI, confidence interval; GMT, geometric mean titer.

[a] log_2.

[b] Immunogenicity population for primary immunization: 33 participants in Group H, 38 participants in Group M, 33 participants in Group L. Immunogenicity population for booster immunization: 32 participants in Group H, 38 participants in Group M, 32 participants in Group L.

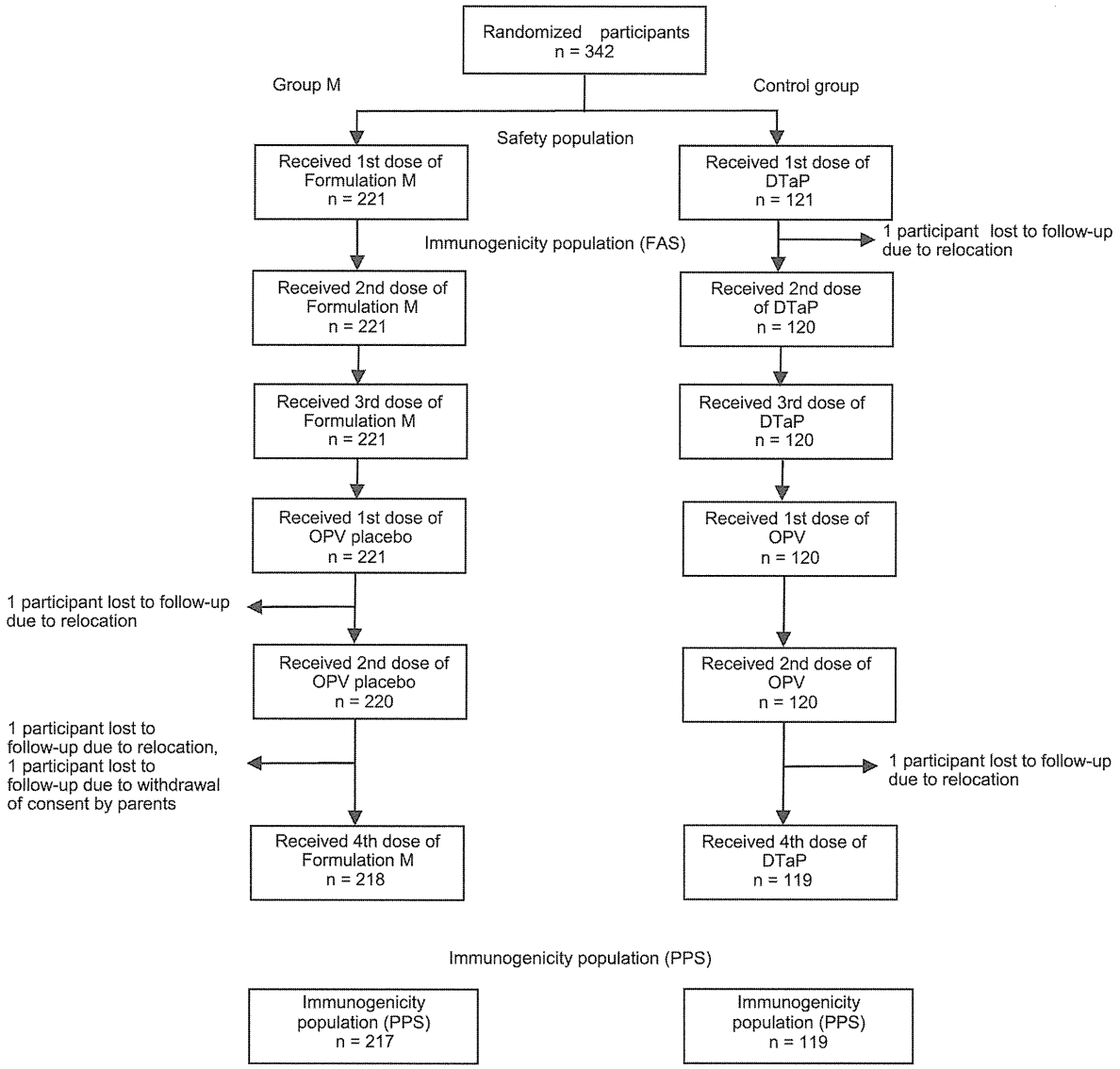

Figure 1. Flow of Phase III study. Abbreviations: DTaP, diphtheria-tetanus-acellular pertussis; FAS, full-analysis set; OPV, oral polio vaccine; PPS, per-protocol set.

the seropositive rate for any component of DTaP, which met the secondary endpoint. The GMTs against each component of DTaP were almost similar between Group M and the Control group, showing that the addition of sIPV did not affect the immunogenicity of DTaP in Formulation M.

Booster Immunization

The seropositive rates and GMTs after booster immunization are shown in Table 4. In Group M, the seropositive rate was 100.0% for all neutralizing antibodies against Sabin types 1, 2, and 3 after booster immunization. After booster immunization, the GMTs (log_2) against Sabin types 1, 2, and 3 were higher in Group M than in the Control group, and they were almost similar among these 3 types in Group M, whereas the GMT against Sabin type 3 tended to be lower in the control OPV group.

The seropositive rate was 100.0% for all components of DTaP in both groups. In addition, the GMTs against each component of DTaP further increased after primary immunization. The GMTs against each component of DTaP were almost the same in Group M and the Control group, showing that the addition of sIPV did not affect the immunogenicity of DTaP in Formulation M.

Safety

Adverse reactions with an incidence of ≥5% in this study are listed in Table 5. The most common adverse reaction was

Table 2. Baseline Attributes of Participants in Phase III Study

	Group M (Formulation M + OPV placebo)		Control Group (DTaP + OPV)	
	n (%)	95% CI (%)	n (%)	95% CI (%)
Participant, no.	221		121	
Sex				
Male	100 (45.2)	38.6–52.1	55 (45.5)	36.4–54.8
Female	121 (54.8)	47.9–61.4	66 (54.5)	45.2–63.6
Age (months)				
Median (min-max)	4 (3–11)	.5–4.6	4 (3–9)	.9–8.2
Preimmunization seropositive rate				
Pertussis				
PT	3 (1.4)	.3–3.9	2 (1.7)	.2–5.8
FHA	13 (5.9)	3.2–9.8	4 (3.3)	.9–8.2
Diphtheria	16 (7.2)	4.2–11.5	6 (5.0)	1.8–10.5
Tetanus	137 (62.0)	55.2–68.4	77 (63.6)	54.4–72.2
Poliovirus				
Sabin strain type 1	104 (47.1)	40.3–53.9	54 (45.0)	35.9–54.3
Sabin strain type 2	78 (35.3)	29.0–42.0	44 (36.7)	28.1–46.0
Sabin strain type 3	20 (9.0)	5.6–13.6	11 (9.2)	4.7–15.8

Group M received Formulation M and OPV placebo, Control group received DTaP and OPV.

Abbreviations: CI, confidence interval; DTaP, diphtheria-tetanus-acellular pertussis; FHA, filamentous hemagglutinin; OPV, oral polio vaccine; PT, pertussis toxoid.

injection-site erythema, but most participants were <Grade 3 (long diameter <5 cm). The most common systemic adverse reaction was pyrexia, but most participants were <Grade 3 (<39.0°C) in both groups. There were no statistically significant differences in the incidence of Grade 3 or more severe pyrexia between the 2 groups. Serious adverse reactions occurred in 2 participants. One participant in Group M was hospitalized due to convulsions that occurred 20 days after the first immunization with Formulation M. The patient recovered well 4 days after hospitalization. The doctor who took care of this patient commented that a relationship between this case and Formulation M was probably very low, however, it could not be completely denied. The other participant, in the Control group, was hospitalized due to pneumonia that occurred 22 days after the third immunization with DTaP. The patient recovered well 4 days after hospitalization. The doctor commented that a relationship could not be completely denied. Neither participant was withdrawn from the study due to the adverse reaction. Based on these results, it is considered that all adverse reactions were clinically acceptable.

DISCUSSION

The Phase II and Phase III clinical studies revealed that the DTaP-sIPV (Formulation M) induced a 100% seropositive rate not only for the 3 types of Sabin strains, but also for the virulent poliovirus strains (type 1: Mahoney strain, type 2: MEF-1 strain, type 3: Saukett strain) after the booster immunization. In the manufacturing of DTaP-sIPV, the addition of sIPV did not affect the immunogenicity of any DTaP component. Formulation M was well tolerated. Based on the immunogenicity and safety data obtained in these clinical studies, this DTaP-sIPV was approved and licensed for use in Japan in July 2012 prior to its worldwide approval.

DTaP-sIPV has been developed with 3 objectives. The first objective is to prevent VAPP while maintaining a high immunity against polio. Theoretically, VAPP will not occur if the OPV is replaced with an sIPV. In addition, the high immunization rate of sIPV is expected to be maintained with DTaP-sIPV by combining an sIPV with DTaP.

The second objective is to prevent paralytic poliomyelitis caused by VDPVs. The spread of VDPVs has been suppressed by enhancing the routine immunization with an OPV [3]. In this study, the titers of neutralizing antibodies against polioviruses induced by the DTaP-sIPV were higher than those induced by OPV. Simply comparing the neutralizing antibody titers may not be sufficient to evaluate the comparative strength of sIPV and OPV against poliovirus, because sIPV and OPV may have different mechanisms for protection against polio. However, it has been shown that neutralizing antibodies play a major role in the protection, considering the efficacy of passive immunization of gamma globulin and active immunization of cIPV to control wild-type polioviruses [22–25]. Therefore, the DTaP-sIPV may also be effective against VDPVs. In fact, transgenic mice immunized with an sIPV were protected against VDPV [26].

The third objective is to prevent paralytic poliomyelitis caused by wild-type viruses. While the eradication of polio caused by wild-type viruses is close to completion, the possibility of polio import cannot be ruled out, given a recent outbreak in China [27]. Therefore, it is very important to understand whether immunity induced by sIPV can prevent polio caused by wild-type polioviruses. The efficacy of DTaP-sIPV should have been evaluated in an efficacy study, but it was impossible to conduct such a study in Japan where polio has already been eradicated. Therefore, in this study we used the neutralizing antibody titer as a surrogate marker.

In the Phase II clinical study, the seropositive rate was 100% for the virulent poliovirus types 1, 2, and 3 after the booster immunization with Formulation M. The neutralizing antibody titer against virulent poliovirus strains was similar to that against corresponding Sabin types 2 and 3, but the titer against the virulent poliovirus type 1 tended to be lower than that

Table 3. Seropositive Rates and Geometric Mean Antibody Titers After Primary Immunization in Phase III Study

Antigen	Group M (Formulation M + OPV placebo) (n = 221)[a] Seropositive Rate (%) (95% CI)	Group M (Formulation M + OPV placebo) (n = 221)[a] GMT (95% CI)	Control Group (DTaP + OPV) (n = 120)[a] Seropositive Rate (%) (95% CI)	Control Group (DTaP + OPV) (n = 120)[a] GMT (95% CI)	*P* value
Poliovirus					
Sabin strain type 1	100.0 (98.3–100.0)	11.02[b] (10.78–11.26)	27.5 (19.7–36.4)	2.41[b] (2.02–2.79)	<.001[c]
Sabin strain type 2	100.0 (98.3–100.0)	10.48[b] (10.32–10.64)	15.0 (9.1–22.7)	1.86[b] (1.57–2.15)	<.001[c]
Sabin strain type 3	100.0 (98.3–100.0)	10.79[b] (10.59–10.99)	7.5 (3.5–13.8)	1.38[b] (1.20–1.56)	<.001[c]
Pertussis					
PT (EU/mL)	98.6 (96.0–99.7)	39.0 (35.5–42.9)	99.2 (95.4–100.0)	39.2 (34.6–44.6)	<.001[d]
FHA (EU/mL)	99.1 (96.7–99.9)	62.0 (56.7–67.7)	100 (96.9–100.0)	77.5 (68.1–88.4)	<.001[d]
Diphtheria (IU/mL)	100.0 (98.3–100.0)	1.72 (1.57–1.89)	100.0 (96.9–100.0)	.982 (.858–1.123)	<.001[d]
Tetanus (IU/mL)	100.0 (98.3–100.0)	1.32 (1.18–1.47)	100.0 (96.9–100.0)	1.27 (1.08–1.48)	<.001[c]

Group M received Formulation M and OPV placebo, Control group received DTaP and OPV. Primary immunization consisted of 3 immunizations. Seropositive is $\geq 1:8(2^3)$.

Abbreviations: CI, confidence interval; DTaP, diphtheria-tetanus-acellular pertussis; EU, enzyme-linked immunosorbent assay units; FHA, filamentous hemagglutinin; GMT, geometric mean titer; IU, international units; OPV, oral polio vaccine; PT, pertussis toxoid.

[a] Immunogenicity population for Sabin polioviruses: 221 participants in Group M, 120 participants in Control group. Immunogenicity population for DTaP components: 217 participants in Group M, 119 participants in Control group.

[b] $(\log_2)$.

[c] Superiority of Group M for Sabin polioviruses: 1-sample binomial test (to verify that null hypothesis [seropositive rate ≤90%] could be rejected).

[d] Noninferiority of Group M to the Control group for DTaP components: Farrington–Manning test (to verify that Group M was not inferior to the Control group by more than 10%) in seropositive rate.

Table 4. Seropositive Rates and Geometric Mean Antibody Titers After Booster Immunization in Phase III Study

Antigen	Group M (Formulation M + OPV placebo) (n = 218)[a] Seropositive Rate (%) (95% CI)	Group M (Formulation M + OPV placebo) (n = 218)[a] GMT (95% CI)	Control Group (DTaP + OPV) (n = 119)[a] Seropositive Rate (%) (95% CI)	Control Group (DTaP + OPV) (n = 119)[a] GMT (95% CI)	*P* value
Poliovirus					
Sabin strain type1	100.0 (98.3–100.0)	12.13[b] (11.93–12.33)	97.5 (92.8–99.5)	11.55[b] (11.10–12.01)	<.001[c]
Sabin strain type2	100.0 (98.3–100.0)	12.61[b] (12.46–12.77)	99.2 (95.4–100.0)	9.62[b] (9.29–9.95)	<.001[c]
Sabin strain type3	100.0 (98.3–100.0)	12.22[b] (12.03–12.42)	83.2 (75.2–89.4)	7.12[b] (6.55–7.69)	<.001[c]
Pertussis					
PT (EU/mL)	100.0 (98.3–100.0)	196 (175–220)	100 (96.9–100.0)	187 (163–214)	<.001[d]
FHA (EU/mL)	100.0 (98.3–100.0)	255 (232–279)	100 (96.9–100.0)	305 (273–342)	<.001[d]
Diphtheria (IU/mL)	100.0 (98.3–100.0)	18.0 (16.3–19.9)	100 (96.9–100.0)	11.9 (10.5–13.6)	<.001[d]
Tetanus (IU/mL)	100.0 (98.3–100.0)	5.4 (4.76–6.12)	100 (96.9–100.0)	4.36 (3.68–5.17)	<.001[d]

Group M received Formulation M and OPV placebo, Control group received DTaP and OPV. Booster immunization was the fourth immunization. Seropositive is $\geq 1:8(2^3)$.

Abbreviations: CI, confidence interval; DTaP, diphtheria-tetanus-acellular pertussis; EU, enzyme-linked immunosorbent assay units; FHA, filamentous hemagglutinin; GMT, geometric mean titer; IU, international units; OPV, oral polio vaccine; PT, pertussis toxoid.

[a] Immunogenicity population for Sabin polioviruses: 218 participants in Group M, 119 participants in Control group. Immunogenicity population for DTaP components: 214 participants in Group M, 118 participants in Control group, whose blood samples were collected.

[b] $(\log_2)$.

[c] Superiority of Group M for Sabin polioviruses: 1-sample binomial test (to verify that null hypothesis [seropositive rate ≤90%] could be rejected).

[d] Noninferiority of Group M for DTaP components: Farrington–Manning test (to verify that Group M was not inferior to Control group by more than 10%) in seropositive rate.

Table 5. Adverse Reactions in Phase III Study

	Group M (Formulation M + OPV placebo)		Control Group (DTaP + OPV)	
Adverse reaction	n (%)	95% CI (%)	n (%)	95% CI (%)
Participants, no.	221		121	
Injection-site reaction				
Injection-site erythema	151 (68.3%)	61.8–74.4	79 (65.3%)	56.1–73.7
≥Grade 3 (long diameter 5 cm)	11 (5.0%)	2.5–8.7	8 (6.6%)	2.9–12.6
Injection-site induration	115 (52.0%)	45.2–58.8	67 (55.4%)	46.1–64.4
Injection-site swelling	69 (31.2%)	25.2–37.8	41 (33.9%)	25.5–43
≥Grade 3 (long diameter 5 cm)	5 (2.3%)	.7–5.2	6 (5.0%)	1.8–10.5
Systemic reaction				
Pyrexia[a]	123 (55.7%)	48.8–62.3	56 (46.3%)	37.2–55.6
≥Grade 3 (39.0°C)	22 (10.0%)	6.3–14.7	13 (10.7%)	5.8–17.7
Diarrhea	91 (41.2%)	34.6–48	40 (33.1%)	24.8–42.2
≥Grade 3 (9 times/day)	4 (1.8%)	.5–4.6	1 (0.8%)	0–4.5
Mood altered	69 (31.2%)	25.2–37.8	26 (21.5%)	14.5–29.9
Rhinorrhea	41 (18.6%)	13.7–24.3	23 (19.0%)	12.4–27.1
Cough	35 (15.8%)	11.3–21.3	15 (12.4%)	7.1–19.6
Rash	28 (12.7%)	8.6–17.8	11 (9.1%)	4.6–15.7
Vomiting	26 (11.8%)	7.8–16.8	16 (13.2%)	7.8–20.6
≥Grade 3 (6 times/day)	2 (0.9%)	.1–3.2	1 (0.8%)	0–4.5
Decreased appetite	26 (11.8%)	7.8–16.8	10 (8.3%)	4–14.7
Pharyngeal erythema	25 (11.3%)	7.5–16.2	8 (6.6%)	2.9–12.6
Productive cough	12 (5.4%)	2.8–9.3	5 (4.1%)	1.4–9.4

Group M received Formulation M and OPV placebo, Control group received DTaP and OPV. Adverse reactions with an incidence of ≥5% in any group are listed.

Abbreviations: CI, confidence interval; DTaP, diphtheria-tetanus-acellular pertussis; OPV, oral polio vaccine.

[a] Defined as axillary temperature of ≥37.5°C.

against corresponding Sabin type 1, although being much higher than the protective level. Subsequently, the neutralizing antibody concentrations (IU/mL) were calculated using the international standard serum provided by the National Institute for Biological Standards and Control for comparison with the previously reported results of clinical studies of cIPV, as shown in Table 6 [28, 29]. The concentration of neutralizing antibody against virulent type 1 induced by Formulation M after the

Table 6. Comparison of Concentration (IU/mL) of Neutralizing Antibodies against Virulent Poliovirus Strains Induced by DTaP-sIPV and cIPVs

	Neutralizing Antibody Concentration (IU/mL, geometric mean)[a] (95% CI)					
	After Primary Immunization[b]			After Booster Immunization		
Vaccine	Type 1	Type 2	Type 3	Type 1	Type 2	Type 3
DTaP-sIPV (Formulation M)	1.5 (1.1–1.9)	10 (7.2–14)	4.4 (3.1–6.2)	9.1 (5.7–14)	240 (150–360)	57 (40–82)
cIPV (IPV-A)[c]	2.1	3.6	5.0	13	25	16
cIPV (IPV-B)[c]	1.3	6.8	3.3	8.0	28	18

Abbreviations: CI, confidence interval; cIPV, conventional IPV produced using virulent polioviruses; DTaP, diphtheria-tetanus-acellular pertussis; IPV, inactivated polio vaccine; IU, international units; OPV, oral polio vaccine; sIPV, IPV using attenuated Sabin strains.

[a] The neutralizing antibody titers (log_2) were converted into the neutralizing antibody concentrations (IU/mL) using international standard serum provided by the National Institute for Biological Standards and Control.

[b] DTaP-sIPV and cIPV were administered in 3 doses and 2 doses, respectively, for primary immunization.

[c] Data were cited from reference No. 28.

primary and booster immunizations was comparable to that after the primary and booster immunizations with a cIPV, indicating that Formulation M is expected to prevent polio caused by wild-type polioviruses as effectively as the cIPV with an established efficacy. In a similar analysis for virulent types 2 and 3, the antibody concentration after the primary and booster immunizations with Formulation M was equal to or higher than that with cIPV, strongly suggesting that DTaP-sIPV is also effective for wild-type polioviruses.

Notes

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Economic evaluation of vaccination programme of 13-valent pneumococcal conjugate vaccine to the birth cohort in Japan

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ABSTRACT

Japan is now preparing to incorporate PCV-7 into the national childhood immunisation programme. Our recently published economic evaluation of using PCV-7 to the birth cohort suggests that the cost to gain one QALY is lower than the WHO's cost-effectiveness criterion for intervention. However, many countries have started to introduce PCV-13 into their national immunisation schedule replacing PCV-7 for preventing pneumococcal diseases among young children. These raise the need to appraise the 'value for money' of replacing PCV-7 with PCV-13 vaccination programme in Japan.

We conducted a cost-effectiveness analysis with Markov model and calculated incremental cost effectiveness ratios (ICERs). Our base-case analyses, which assumed both PCVs have no net indirect effect and set the cost of PCV-7/PCV-13 per shot at ¥10,000 (US$125)/¥13,000 (US$163).

The results show that in Base-case A (assumed PCV-13 has no additional protection against AOM compared to PCV-7), replacing PCV-7 with PCV-13 will cost ¥37,722,901 (US$471,536) or ¥35,584,455 (US$444,850) per QALY when the caregiver's productivity loss is not included or is included, respectively. While in Base-case B (assumed PCV-13 has additional protection against AOM compared to PCV-7), ¥343,830 (US$4298) per QALY or more QALY is gained by saving money without or with caregiver's productivity loss, respectively.

We also find that, in Base-case B if cost per PCV-13 shot is equal to or less than that ¥17,000, then a PCV-13 vaccination programme offered to the birth cohort in Japan is likely to be a socially acceptable option compared to the current PCV-7 vaccination programme. Furthermore, if cost per PCV-13 shot is equal to or less than ¥12,000, replacing PCV-7 with PCV-13 will save money and gain more QALYs. While in Base-case A, the replacement can only be socially acceptable if cost per PCV-13 shot is equal to or less than ¥11,000.

1. Introduction

The 7-valent pneumococcal conjugate vaccine (PCV-7) was firstly approved in the USA in 2000 for the prevention of diseases caused by *Streptococcus pneumoniae* (pneumococcus) among infants and young children. In 2007, WHO recommended the vaccine to be incorporated into national childhood immunisation programmes in every country [1]. In 2009, two pneumococcal vaccines with extended serotype coverage, 10-valent (PCV-10) and 13-valent (PCV-13), were introduced, and since then, they have been gradually replacing PCV-7 [2]. The PCV-10 conjugates to non-typable *Haemophilus influenza* carrier protein, while PCV-13 conjugates to the same carrier protein (CRM197) as PCV-7. In order to support the adoption of PCV-10 or PCV-13, cost-effectiveness studies have been performed in various countries. In general, results of these studies have demonstrated that the use of PCV-10 or PCV-13 is cost-effective or cost saving compared to PCV-7 vaccination programme in prevention of the disease caused by *S. pneumoniae* [3–6].

In Japan, PCV-7 was approved on October 26, 2009. The government disbursed a budget to encourage municipalities in launching a public 3+1 dose vaccination programme (3 primary doses and 1 booster dose) on November 26, 2010, which will continue until March 31, 2013. Therefore, currently all municipalities give subsidies to PCV-7 vaccinees. The attainable vaccination rate for Japanese infants is considered to be about 60%, according to the "Provisional Special Fund for the Urgent Promotion of Vaccination against Such Diseases as Cervical Cancer" by the government in 2011. A study group of ten health institutions and affiliated paediatricians have reported that there was a decrease in invasive pneumococcal diseases (from 333 cases in 2010 to 113 cases in 2011) after the introduction of PCV-7 [7]. As to PCV-10 and PCV-13, both are not yet available in Japan, while the latter is now under the process of approval and experts have expressed their expectations

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[8]. The possible availability of PCV-13 raises the need to evaluate effective ways in protecting the birth cohort from pneumococcal-related diseases in Japan.

It is said that there are five hurdles to overcome in the diffusion process of new health intervention: quality, safety, efficacy, cost-effectiveness and affordability [9]. In regards to the cost-effectiveness and affordability of PCV-7 vaccination programme, our recently published economic evaluation of using PCV-7 to the birth cohort in Japan suggests that if we adopt WHO's criterion that an intervention is 'cost-effective' if ICER (in QALY) is between 1 and 3 times of GDP per capita, then PCV-7 vaccination programme would be an efficient use of finite resources in healthcare from the societal perspective, regardless of the co-payment level [10]. Furthermore, the study shows that if full subsidy is provided for the vaccination programme, the level of budget impact is less than ¥11,000,000 (US$137,500; US$1 = ¥80, based on the average exchange rate of 2012) for a municipality with 1000 birth cohort in the 1st year, and 2nd to 5th year birth cohort proportional to the birth cohort population of estimated future population.

This study aims to investigate the cost-effectiveness of replacing the current 3 + 1 dose schedule of PCV-7 vaccination programme with 3 + 1 dose schedule of PCV-13 vaccination programme in Japan, foreseeing the possible replacement after the approval of PCV-13. The results should deepen our understanding about the implications of preventing pneumococcus-caused diseases among infants and young children to healthcare financing and inform policy makers of Japan as well as in other developed countries. Since PCV-10 is not even under the process of approval, it is not considered as an alternative in this study, however, if in such case, we may evaluate with appropriate comparator, either PCV-7, PCV-13, other PCV products [11], or all.

2. Method

We conduct a cost-effectiveness analysis with Markov modelling from the societal perspective. The Markov model is from our previous study [10], while epidemiological data and resource use are updated. Japanese data sources are reviewed together with international literature to parameterise the model.

2.1. Programmes and Markov model

We define two vaccination programmes: current PCV-7 programme and the possible replacement, i.e., PCV-13 programme, with the same vaccination schedule (3 + 1). We assume that vaccination is fully subsidised for the uptake of 4 doses of either PCV-7 or PCV-13. The average vaccine uptake rate for both vaccination programmes is set as 76.1%, which is the rate of DPT vaccination programme in 2010 [12]. This rate is adopted for two reasons: first, it has been a mere 2 years since the introduction of PCV-7 in Japan, and no adequate data is yet available to estimate its uptake rate; secondly, the vaccination schedule of DPT is similar to that of PCV-7 and PCV-13. We also assume that among the vaccinees of first 3 doses, 13.8% will uptake PCV-7/PCV-13 alone, while 23.1% will uptake simultaneously with Hib vaccine, and 63.1% will uptake simultaneously with one other vaccine listed in the national immunisation schedule [13]. In regards to the 4th dose, 40% will uptake PCV-7/PCV-13 alone, and 60% will uptake simultaneously with one other listed vaccine [13].

We then consider about the municipality's decision in launching a 5-year programme, which is assumed for reconsideration or redesigning of the programme, as it is often employed in organising public health programmes in Japan [14]. The birth cohorts of 5 years used in the model are from Population estimates of Japan [15]. Incremental cost-effectiveness ratios (ICERs) of PCV-13 programme to PCV-7 programme are calculated to determine the efficiency of the resource use.

The disease model of the health effects of pneumococcal vaccination includes the possibility of subsequent pneumococcal disease, such as: bacteraemia (including sepsis), meningitis, all-cause hospitalised pneumonia, acute otitis media (AOM, including simple and complex), sequelae after meningitis, and death from or other than the related diseases in the model (Fig. 1). A Markov cycle for each stage is set at 1 year. The time frame is 5 years after the entering of a birth cohort because the diseases caused by *S. pneumoniae* decrease significantly among children aged 5 years and over [16]. Life expectancy of survived patient with or without neurological sequelae is assumed as 53.9 years or to have a life expectancy of Japanese population, respectively [10]. Adverse effects associated with vaccination are not considered because those of PCV-13 are similar to those of PCV-7 [17–19].

2.2. Outcomes estimation

Outcomes in terms of years of life saved (YOLS) and quality adjusted life year (QALY) are estimated by assigning transition probabilities and utility weights from the literature to the Markov model.

2.3. Annual incidence rates and case fatality rates

Annual incidence rates of meningitis and of bacteraemia among children younger than 5 years old without vaccination are derived from a 3-year (2007–2009) nationwide survey by Kamiya [20]; of AOM are computed by the AOM episodes by child [21], multiplied by the "proportion of clinically diagnosed AOM episodes due to pneumococcus (34.1%)" [22]; of hospitalised community acquired pneumonia (CAP) are from a retrospective study of 18 hospitals with paediatric wards in Chiba city, Japan [23]. Proportions of meningitis that resulted in hearing impairment or neurological sequelae are from Kamiya [20] and Iwata et al. [24]. Case fatality rates of meningitis and of bacteraemia are also from Kamiya [20]; of hospitalised pneumonia are estimated from Patient survey [25] and Vital statistics [26]. Deaths from causes other than the above diseases are also from the Vital statistics [26]. All these rates are shown in Table 1.

2.4. Vaccine effectiveness

2.4.1. Direct effect

The vaccine effectiveness (VEs) of PCV-7 against vaccine-serotype-IPD (including bacteraemia and meningitis), vaccine-serotype-AOM, and hospitalised radiograph-confirmed pneumonia among children under 2 years old are 80%, 54%, and 27%, respectively, based on the systematic review reported by the Cochrane Collaboration [27,28]. The VEs of PCV-13 are not available at the time of this study. Based on the immunogenicity data, we assume PCV-13 is as immunogenic as PCV-7 for common serotypes and has comparable levels of antibody for serotypes unique to PCV-13 [2,18,19]. Proportion of IDP episodes due to PCV-7/PCV-13 serotype is assumed as 68.5%/80.9% [7]; of hospitalised community acquired pneumonia (CAP) episodes, 66.7%/81.0% [23]; of AOM episodes, 68.2%/86.0%, for 0 to <3 years old, and 48.5%/77.9% for 3 to <5 years old [29]. The VEs against IPD and AOM are of specific vaccine serotypes only, therefore, they are multiplied by the proportion of relevant disease episodes due to PCV-7/PCV-13 serotypes to adjust to our disease model, while the VEs against hospitalised pneumonia are not of specific vaccine serotypes, and therefore there is no need to adjust. For those aged over 2 and under 5 years, the VEs against IPD and hospitalised pneumonia are assumed to decline

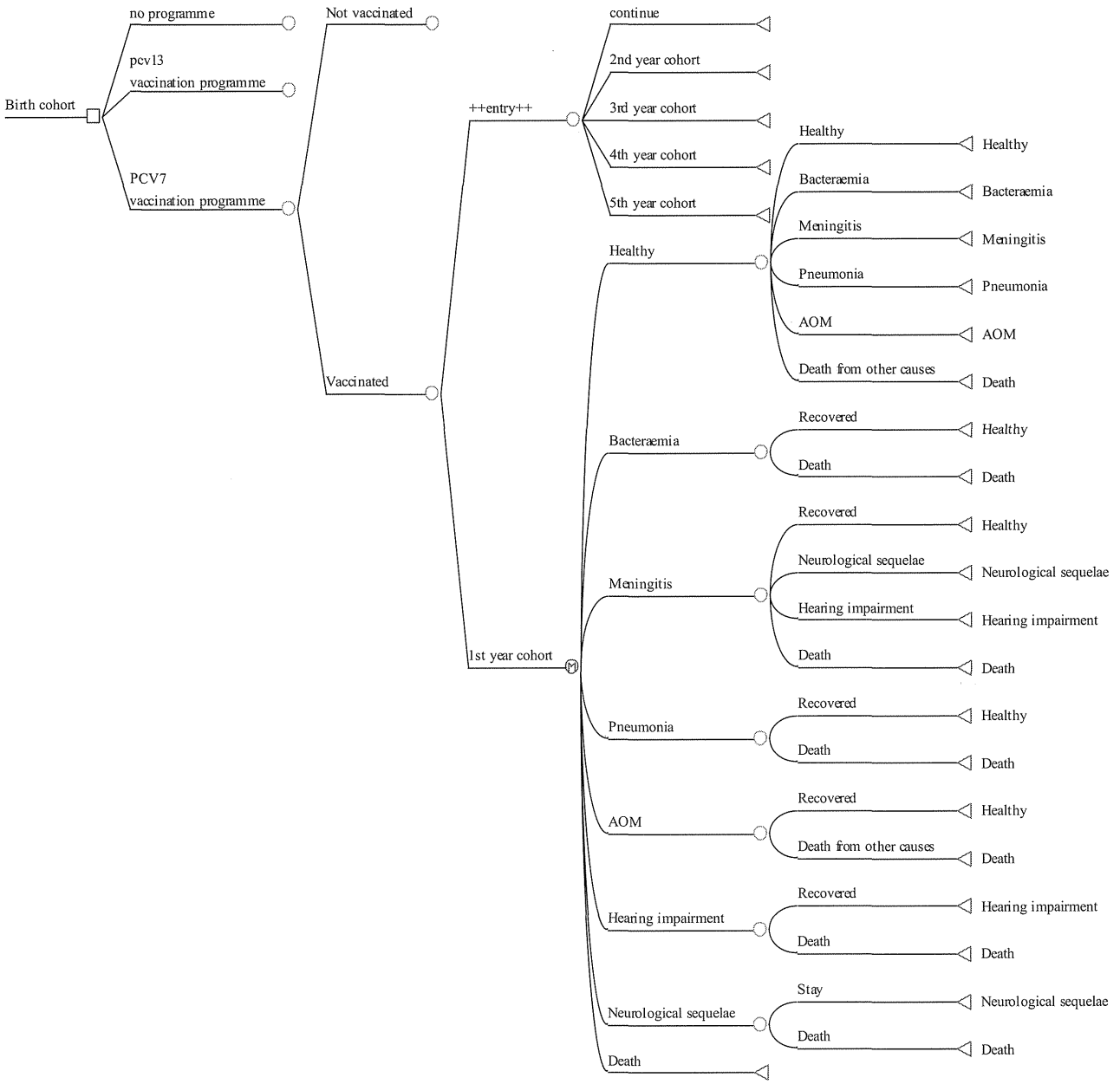

Fig. 1. Markov model. 1st to 5th year birth cohorts are from population estimates of Japan. "Healthy" means being without the diseases defined by the model. The "++entry++" indicates new birth cohort which falls into programmes during the 2nd to 5th year after the start of the vaccination programme. No programme is shown as reference.

by 3% annually for both PCV-7 and PCV-13 [30]. All these data are shown in Table 1.

No efficacy data against otitis media were available for serotypes 1, 3, 5, 6A, 7F, and 19A from the package insert of Prevenar 13® (brand name of PCV-13) sold in the US [31]. Therefore, we set two base-cases for analyses: "Base-case A", which assumes that the prevention of AOM by PCV-13 is limited to the seven serotypes of PCV-7 only; and "Base-case B", which assumes that the prevention of AOM by PCV-13 is straightforwardly extended to cover non-PCV-7 serotypes.

2.4.2. Indirect effects

We do not consider the net indirect vaccine effect (herd protection minus serotype replacement effect) in our base-case analysis, but conduct four scenario analyses by assuming different net indirect effects among children aged under 5 years old as observed in European countries and the US. Assumptions made for each scenario and two base-cases are shown in Table 2. The net indirect effect in non-vaccinated children older than 5 years old is not considered in the scenario analyses because of the discrepancies among reports from previous studies [32]. In the US, indirect effects was observed among adults after the nationwide implementation of PCV-7 in 2000, while in European countries, such as Spain, France, and the UK, no overall reduction of IPD incidences were observed among adults even after three years of introduction in routine vaccination [32]. Rozenbaum indicates that possible factors responsible for these differences may include the vaccine-serotype coverage, and/or implemented vaccination schedules, and/or antibiotic resistance rates, and/or pneumococcal disease incidences prior to vaccination.

2.5. Costing

From the societal perspective, costing should cover the opportunity costs borne by various economic entities in the society [33]. In the context of this study, the amount of direct payments costs

Table 1
Epidemiological data used on model.

Variable		Reference
Vaccine uptake rate	76.1%	[12]

Variable	Total	Male	Female	Reference
Population of birth cohort entering the model, unit: 1000				[15]
1st year	1004	515	489	
2nd year	981	504	478	
3rd year	954	489	464	
4th year	926	475	451	
5th year	900	462	438	

Variable	Age groups					Reference
	0 to <1	1 to <2	2 to <3	3 to <4	4 to <5	
Annual incidence rates per 100,000 population						
Invasive pneumococcal disease cases: meningitis	7.46	3.83	1.11	0.94	0.27	[20]
Invasive pneumococcal disease cases: bacteraemia	15.2	45.4	12.0	5.8	4.0	[20]
Clinically diagnosed AOM episodes	103,100	113,881	67,160	52,589	40,371	[21]
All-causes hospitalised CAP	1760	1760	1760	1760	1760	[23]
Proportion of meningitis that results to hearing impairment, %	3.3	3.3	3.3	3.4	3.4	[20,24]
Proportion of meningitis that results to neurological sequelae, %	15.7	15.7	15.7	15.6	15.6	[20,24]
Proportion of clinically diagnosed AOM episodes due to pneumococcus, %	34.1	34.1	34.1	34.1	34.1	[22]
Case fatality rate, %						
Bacteraemia	0.04	0.04	0.04	0.04	0.04	[20]
Meningitis	0.21	0.21	0.21	0.21	0.21	[20]
Hospitalised pneumonia	0.11	0.11	0.11	0.11	0.11	[25,26]
Vaccine effectiveness (VE) of PCV-7[a], %						
In reducing vaccine serotype IPD	80.0	80.0	77.6	75.3	73.0	[27]
In reducing vaccine serotype AOM	54.0	54.0	572.4	50.8	49.3	[27]
In reducing hospitalised pneumonia	27.0	27.0	26.2	25.4	24.6	[28]
Proportion of IPD episodes due to PCV-7 serotype, %	68.5					[7]
Proportion of IPD episodes due to PCV-13 serotype, %	80.9					[7]
Proportion of hospitalised CAP episodes due to PCV-7 serotype, %	66.7					[23]
Proportion of hospitalised CAP episodes due to PCV-13 serotype, %	81.0					[23]
Proportion of AOM episodes due to PCV-7 serotype, %	68.2	68.2	68.2	48.5	48.5	[29]
Proportion of AOM episodes due to PCV-13 serotype, %	86.0	86.0	86.0	77.9	77.9	[29]

Variable		Reference
Life expectancy of neurological sequelae	53.9	[10]
Life expectancy of Japanese population at age 5	74.9 male; 80.8 female	[10]
Utility weight		[10]
Healthy	1	
Hearing impairment	0.9	
Neurological sequelae	0.57	
Curable bacteraemia	0.9921	
Curable meningitis	0.9768	
Curable pneumonia	0.994	
AOM	0.995	
Death	0	

[a] VEs of PCV-13 are assumed to be as immunogenic as PCV-7 for common serotypes and has comparable levels of antibody for serotypes unique to PCV-13.

borne by municipal authorities, vaccinees, patients and social insurers are considered, while indirect costs of vaccination programme are not included, because it is assumed that the programme is built within the public health services infrastructure. Therefore, costs of vaccination, treatment costs of pneumococcal-related diseases and costs associated to care-giver's lost productivity, such as accompanying a child for vaccination, for medical treatment, or to take care of a child with sequelae, are counted. Productivity loss due to mortality or morbidity is not included, as including this into cost-effectiveness analysis may be argued as double counting while survived cases are incorporated in the utility weights and disease duration in calculating QALYs [33].

2.5.1. Direct medical costs

The vaccination cost per a shot of PCV-7 is assumed at ¥10,000 (US$125) [10]; per a shot of PCV-13 is assumed at 1.3 times that of PCV-7 based on the report from "The Pharma Letter" [34]. Treatment costs per episode of survived/fatal bacteraemia, survived/fatal meningitis, and long-term treatment costs for an individual with hearing impairment or neurological sequelae are

Table 2
Base-cases and scenarios.

Base-case A	No net indirect effect. Not effective against serotypes unique to PCV-13 (1, 3, 5, 6A, 7F, 19A)
Base-case B	No net indirect effect. Effective against serotypes unique to PCV-13 (1, 3, 5, 6A, 7F, 19A)
Scenario-1	Net indirect effect to non-vaccinated aged under 5. The effect to IPD only. No net indirect effect to aged over 5.
Scenario-2	Net indirect effect to non-vaccinated aged under 5. The effect to IPD, hospitalised pneumonia. No net indirect effect to aged over 5.
Scenario-3	Net indirect effect to non-vaccinated aged under 5. The effect to IPD, AOM. No net indirect effect to aged over 5.
Scenario-4	Net indirect effect to non-vaccinated aged under 5. The effect to IPD, hospitalised pneumonia, AOM. No net indirect effect to aged over 5.