Table 3
Costs.

Variable		Reference
Cost per PCV-7	¥10,000	[10]
Cost per PCV-13	¥13,000	[34]

	Age groups					Reference
	0 to <1	1 to <2	2 to <3	3 to <4	4 to <5	
Treatment cost						
Bacteraemia episode, survive	¥419,153	¥419,153	¥419,153	¥392,802	¥392,802	[24]
Bacteraemia episode, death	¥1,032,126	¥1,032,126	¥1,032,126	¥1,010,205	¥1,010,205	[24]
Meningitis episode, survive	¥852,642	¥852,642	¥852,642	¥843,867	¥843,867	[24]
Meningitis episode, death	¥1,470,421	¥1,470,421	¥1,479,196	¥1,510,669	¥1,510,669	[24]
Pneumonia episode	¥221,133	¥221,133	¥221,133	¥164,916	¥164,916	[35]
AOM episode	¥31,990	¥35,313	¥61,927	¥43,659	¥44,359	[21]
Hearing impairment (long term treatment)/year	¥79,422	¥79,422	¥79,422	¥78,057	¥78,057	[21]
Neurological sequelae (long term treatment)/year	¥420,464	¥420,464	¥420,464	¥380,671	¥380,671	[21]
Variables related to care-giver's productivity loss						
Frequency of outpatient visits/number of hospitalisation days						
Bacteraemia episode	2.9 visits/11.5 days			2.8 visits/10.5 days		[21]
Meningitis episode	8.1 visits/22.7 days			7.8 visits/21.1 days		[21]
Pneumonia episode	2.7 visits/6.8 days			2.8 visits/4.9 days		[35]
Hearing impairment	8 h per day until the child is admitted to special support education system	[21]				
Neurological sequelae						
Absent working hours per AOM episode (h)	33.6	27.7	50.3	43.1	39.4	[21]
Average hourly wage of Japanese women labourers	¥1328					[36]

US$1 = ¥80.

according to Iwata et al. [24]. Treatment costs per episode of pneumonia caused by *S. pneumoniae* are according to Ishiwada et al. [35]. Treatment costs per episode of AOM are the weighted average of simple and complex cases reported by Yamanaka et al. [21]. The proportions of complex cases are: 37%, 49%, 25%, 19%, and 14%, for children aged 0 to <1, 1 to <2, 2 to <3, 3 to <4, and 4 to <5, respectively [21]. All these costs are shown in Table 3.

2.5.2. Productivity loss by care-giver

Under the context of this study, productivity loss per disease episode or per shot is valued as a product of care-giver's absent working hours from paid employment (8 working hours/day) and an average hourly wage, ¥1328 (US$17), of Japanese women labourers [36]. Productivity loss of a care-giver to accompany a child for one uptake of vaccine is assumed as a half of a day (4 h) when uptaking PCV-7 or PCV-13 alone, $1/2 \times 4$ h when uptaking simultaneously with Hib vaccine. $1/2 \times 4$ h is assumed because Hib vaccination programme was introduced on the same day as PCV-7 vaccination programme in Japan, and therefore, 4 h of productivity loss should be shared equally in simultaneous uptake of PCV-7/PCV-13 and Hib vaccine. And 0 h, when uptaking simultaneously with one other listed vaccine, because it can be assumed that no incremental productivity loss occurs to uptake PCV-7/PCV13 in particular. As to the productivity loss per disease episode, the frequency of outpatient visits and the number of hospitalisation days of a meningitis episode are from Yamanaka et al. [21]; of a pneumonia episode are from Ishiwada [35]. We assume 4 absent working hours for one outpatient visit and 8 absent working hours for one hospitalised day. The average absent working hours of an AOM episode are the weighted average of simple and complex AOM derived from Yamanaka et al. [21]. We assume that the absent working hours of a care-giver to take care of one child with hearing impairment or neurological sequelae is 8 h per day until the child is admitted to special support education system, which is at age 6 in Japan.

2.6. Discounting

Costs and outcomes were discounted at a rate of 3% [33].

2.7. Scenario analyses, sensitivity analyses, and probabilistic analyses

In order to assess the impact of herd effects on outcomes of PCV-7/PCV-13 vaccination, scenario analyses, which assume four different net indirect effects in non-vaccinated children aged under 5 years old (Table 2), are performed: Scenario-1 limits the herd effect to IPD only; Scenario-2 extends the effect to IPD and hospitalised pneumonia; Scenario-3 extends the effect to IPD and AOM; and Scenario-4 assumes the effect to all the diseases, i.e., IPD, hospitalised pneumonia and AOM. We assume the protection resulted from herd effects would be as effective as direct effects of vaccination, based on the report from the US [37]. One-way sensitivity analyses are performed on cost of one shot of PCV-13 as well as on the VEs of PCV-7 and PCV-13, which several studies have reported to have a significant impact on the results. For a cost of one shot of PCV-13, lower and upper values are set at ¥10,000 (US$125, equal to the current cost of PCV-7) and ¥20,000 (US$250, double the current cost of PCV-7), respectively. For the VEs of PCV-7, the lower value is changed by −20%, while the upper value is set equal to the VEs of PCV-13. On the other hand, for the VEs of PCV-13, the upper value is changed by +20%, and the lower value is set equal to the VEs of PVC-7. Sensitivity analyses on epidemiological data, life expectancy, utility weights and treatment costs of disease episodes are omitted because these are assumed as similar in both PCV vaccination programmes.

We also conduct a thousand times Monte Carlo simulation, i.e., probabilistic analyses, for which VEs are assumed to have an equilateral triangle distribution corresponding to the range tested in one way sensitivity analyses. Other variables are fixed at their base-case values.

3. Results

3.1. Avoided cases

The estimated disease cases avoided by PCV-7/PCV-13 vaccination programme compared with no programme for 100,000 birth cohort in the 5-year period are as follow: 8.2/9.7 cases of meningitis,

Table 4
Results of Base-case analyses.

	Base-case A: PCV-13 with no additional VE to PCV-7 on AOM[a] Cost per child				Base-case B: PCV-13 with additional VE to PCV-7 on AOM Cost per child			
	Vaccine cost	Diseases treatment costs	Productivity loss (uptake vaccine)	Productivity loss (disease treatment)	Vaccine cost	Diseases treatment costs	Productivity loss (uptake vaccine)	Productivity loss (disease treatment)
No programme	¥0	¥64,346	¥0	¥62,931	¥0	¥64,346	¥0	¥62,931
PCV-7	¥28,725	¥49,747	¥4414	¥47,924	¥28,725	¥49,747	¥4414	¥47,924
PCV-13	¥37,342	¥48,975	¥4414	¥47,479	¥37,342	¥41,507	¥4414	¥38,646

	Base-case A: PCV-13 with no additional VE to PCV-7 on AOM[a] Effect per child		Base-case B: PCV-13 with additional VE to PCV-7 on AOM Effect per child	
	QALY	YOLS	QALY	YOLS
No programme	32.8087	32.8152	32.8087	32.8152
PCV-7	32.8109	32.8158	32.8109	32.8158
PCV-13	32.8111	32.8160	32.8120	32.8160

	Base-case A: PCV-13 with no additional VE to PCV-7 on AOM[a]				Base-case B: PCV-13 with additional VE to PCV-7 on AOM			
	Cost/QALY	Cost/QALY	Cost/YOLS	Cost/YOLS	Cost/QALY	Cost/QALY	Cost/YOLS	Cost/YOLS
CER/ICER	Without productivity loss	With productivity loss	Without productivity loss	With productivity loss	Without productivity loss	With productivity loss	Without productivity loss	With productivity loss
PCV-7 vs. No programme	¥6,352,110	¥1,588,575	¥23,512,220	¥5,880,083	¥6,352,110	¥1,588,575	¥23,512,220	¥5,880,083
PCV-13 vs. No programme	¥9,034,940	¥4,495,903	¥29,476,620	¥14,667,948	¥4,368,278	Cost less, gain more	¥19,457,218	Cost less, gain more
PCV-13 vs. PCV-7	¥37,722,901	¥35,584,455	¥54,261,241	¥51,185,265	¥343,830	Cost less, gain more	¥2,606,959	Cost less, gain more

[a] Based on the package insert of Prevenar®.

Table 5
Results of scenario analyses.

	PCV-13 vs. PCV7	
	Cost/QALY Without productivity loss	Cost/QALY With productivity loss
Base-case A	¥37,722,901	¥35,584,455
Scenario-1	¥33,661,992	¥31,387,802
Scenario-2	¥27,824,591	¥25,683,001
Scenario-3	¥31,387,702	¥31,387,802
Scenario-4	¥25,682,885	¥25,683,001
Base-case B	¥343,830	Cost less, gain more
Scenario-1	¥308,676	Cost less, gain more
Scenario-2	¥115,860	Cost less, gain more
Scenario-3	Cost less, gain more	Cost less, gain more
Scenario-4	Cost less, gain more	Cost less, gain more

49.4/58.4 cases of bacteraemia, 1739.4/2112.9 cases of hospitalised pneumonia, 66,188/66,188 (Base-case A) or 72,728 (Base-case B) of AOM, and 1.86/2.26 cases of death due to either meningitis, bacteraemia or pneumonia. If PCV-13 replaces PCV-7, the estimated incremental number of avoided cases will be: 1.49 of meningitis, 8.94 of bacteraemia, 373.5 of hospitalised pneumonia, none or 6540.2 of AOM in Base-case A or Base-case B, respectively, and 0.40 cases of death due to either meningitis, bacteraemia or pneumonia. The reduced disease cases resulting from replacing PCV-7 with PCV-13 would be 18.1%, 21.5%, and 9.9%, for IPD, hospitalised pneumonia, and AOM, respectively.

3.2. Cost, effectiveness, and cost-effectiveness

Given the purpose of this study, the description should focus on the comparison between PCV-13 programme and PCV-7 programme. The results of the comparison between PCV-7/PCV-13 against no-programme are shown in Table 4 as reference.

Table 4 shows the results of base-case analyses. When comparing PCV-13 programme with PCV-7 programme, estimated average incremental effects per child are 0.0002QALY/0.0001YOLS for Base-case A, and 0.0011QALY/0.0001YOLS for Base-case B. In terms of QALY gained, IPD contributed 7.1%, hospitalised pneumonia contributed 11.9%, and AOM contributed 81.0% to the figures (in Base-case B).

PCV-13 programme reduces both disease treatment costs and care-giver's productivity loss due to disease treatment. However, when the care-giver's productivity loss is not included, the reduced disease treatment costs alone do not offset the vaccination cost, which means that the vaccination programme turns out to be 'gain more but cost more'. Estimated ICERs are ¥37,722,901 (US$471,536) per QALY gained or ¥54,261,241 (US$678,266) per YOLS gained for Base-case A; ¥343,830 (US$4298) per QALY gained or ¥2,606,959 (US$32,587) per YOLS gained for Base-case B. When the care-giver's productivity loss is included, the ICERs are ¥35,584,455 (US$444,806) per QALY gain or ¥51,185,265 (US$639,816) per YOLS gain for base-case A. While for Base-case B, the sum of reduced disease treatment costs and reduced caregiver's productivity loss outweighs the vaccination cost. It can be concluded that PCV-13 programme not only gains more QALY/YOLS but also saves money compared to PCV-7 programme.

3.3. Uncertainty analyses

Table 5 shows the results of eight scenario analyses. ICER decreases as expected from indirect effect. In Base-case A, it decreases from ¥37,722,901 (US$471,536) to ¥33,661,992 (US$420,775) in Scenario-1; to ¥27,824,591 (US$347,807) in Scenario-2; to ¥31,387,702 (US$392,346) in Scenario-3; and to

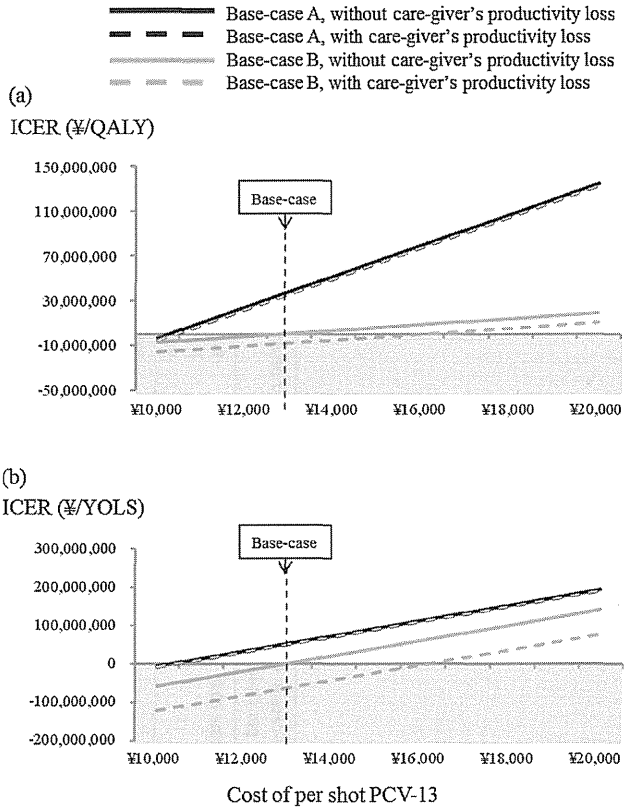

Fig. 2. The effect on ICERs by changing cost of PCV-13. The grey area shows cost saving, i.e., cost less and gain more.

¥25,682,885 (US$321,036) in Scenario-4; per QALY, when care-giver's productivity loss is not included. In Base-case B, it decreases from ¥343,830 (US$4298) to ¥308,676 (US$3858) in Scenario-1; to ¥115,860 (US$1448) in Scenario-2; cost less and gain more in both Scenario-3 and Scenario-4; per QALY, when caregiver's productivity loss is not included. It consistently costs less and gains more in all eight scenarios when caregiver's productivity loss is included.

Fig. 2(a) and (b) shows how the ICER of PCV-13 programme varies with changing costs per shot compared to PCV-7 programme. PCV-13 dominates (costs less and gains more) PCV-7 at cost per shot equal to or lower than that of PCV-7, i.e., ¥10,000 (US$125) in Base-case A regardless of care-giver's productivity loss, and regardless of measuring QALY or YOLS; While in Base-case B, ¥12,000 (US$150) or ¥16,000 (US$200), when care-giver's productivity loss is included or not included, respectively.

Fig. 3 shows the results of one-way sensitivity analyses performed on VEs which decrease or increase the ICER more than ¥500,000 (US$6250) per QALY. The top 10 variables are all related to VE against AOM. Among the variables, the VE of PCV-13 against AOM (1–2 years old) shows the largest impact on the result. The upper/lower value of this variable decrease/increase the ICER by ¥894,798 (US$11,185)/¥887,925 (US$11,099) per QALY, which is −260%/+258% of the ICER of the base-case.

Fig. 4 presents four cost-effectiveness acceptability curves (CEACs) estimated by the probabilistic sensitivity analyses: For Base-case A, when productivity loss is included/not included, the probability of ICER to be less than ¥5,000,000 (US$62,500) per QALY is 3.8%/0.1%, respectively. For Base-case B, when productivity loss is included/not included, the probability that PCV-13 programme dominates PCV-7 programme is 99.0%/42.5%, and the probability of ICER to be less than ¥5,000,000 (US$62,500) per QALY is 99.9%/95.0%.

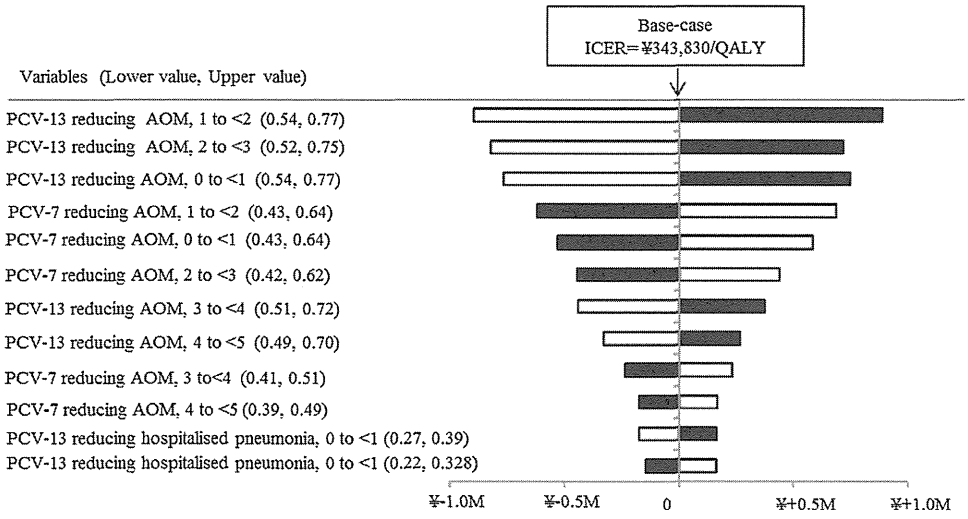

Fig. 3. Sensitivity analyses performed on vaccine effectiveness.

4. Discussion

We estimate the cost-effectiveness of replacing the current PCV-7 vaccination programme with PCV-13 vaccination programme, and the effectiveness of PCV-13 is calculated based on the effects of PCV-7 and the serotype coverage of PCV-13 compared to PCV-7, as done in other studies [4–6].

Our base-case analyses, which sets the cost of PCV-13 per shot at 1.3 times that of PCV-7 (¥13,000/US$163), shows 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 an additional ¥37,722,901 (US$471,536) or ¥35,584,455 (US$444,850) per additional 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 additional QALY or more QALY is gained by saving money without or with caregiver's productivity loss, respectively.

Sensitivity analyses on cost of one shot of PCV-13 show that in Base-case A, if the cost of one shot of PCV-13 is equal to that of PCV-7, i.e., ¥10,000 (US$125), replacing PCV-7 with PCV-13 will save money and gain more QALY or YOLS regardless of caregiver's productivity loss. At cost equal to or less than 11,000 (US$138), ICER will be lower than ¥10 million (US$125,000) per QALY regardless of caregiver's productivity loss. While in Base-case B, at ¥12,000 (US$150)/¥16,000 (US$200) per shot, the replacement will save money and gain more QALY or YOLS regardless of caregiver's productivity loss.

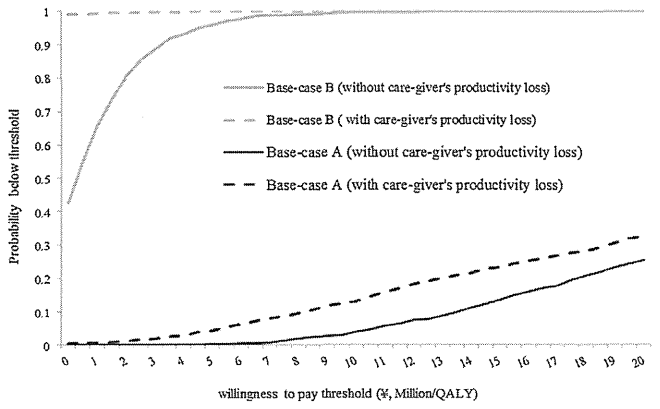

Fig. 4. Cost-effectiveness acceptability curves (CEACs) for base-cases with/without care-giver's productivity loss. CEAC is a commonly used visual aid for communicating the results of probabilistic sensitivity analysis in cost-effectiveness models, which presents relative cost-effectiveness as a function of the threshold ICER. The graphed value of any comparator at a particular willingness-to-pay represents the probability that it is cost-effective, based on the uncertainties included in the simulation.

Sensitivity analyses on VEs performed on Base-case B show that the VE of PCV-13 against AOM (1 to <2 year) has the largest impact on the result, with its lower/upper value increasing/decreasing the ICER about ¥9,000,000 (US$11,250) per QALY.

The probabilistic sensitivity analyses show that the probabilities of PCV-13 programme to be under ¥5,000,000 (US$62,500) per QALY are 0.1% (Base-case A, care-giver's productivity loss not included) to 99.9% (Base-case B, care-giver's productivity loss included).

In Base-case B, the ICERs in QALY of our base-case analyses, scenario analyses and sensitivity analyses are all less than a willingness-to-pay threshold suggested for healthcare intervention, i.e., ¥5,000,000 (US$62,500) per QALY gained [38], and are under WHO's cost-effective criterion for intervention, i.e., less than 3 times of GDP per capita (≒¥11,000,000 or US$137,500 in Japan) [39]. Therefore, when we consider the "value for money", the replacement of PCV-7 with PCV-13 vaccination programme would be a socially acceptable option in Japan from the viewpoint of health economics. On the other hand, in Base-case A, unless the cost of one PCV-13 shot is equal to or less than ¥11,000 (US$138), the ICERs would all be over ¥11,000,000 (US$137,500). Therefore the replacement is not considered a socially acceptable option in Japan.

A recent study reported the cost-effectiveness ratio (CER) of Rotavirus vaccination programme in Japan, of which ratio was ¥9.8 million per QALY [40]. This is larger than our CERs of PCV-7 or PCV-13, which is ¥6.4 million or ¥9.0 million per QALY, respectively. Several studies from overseas reported on the cost-effectiveness of introducing PCV-13. Among them, some compared PCV-7 and PCV-13 with no-programme from the societal perspective and found PCV-13 is more cost-effective than PCV-7 with or without considering net-indirect effect [6,32]. By taking the cost-effective ratios (CERs) of PCV-7/PCV-13 vaccination programme and comparing them to that of no-programme, our study yields a result that is consistent with those previous studies. On the other hand, some studies evaluated the transition of PCV-7 to PCV-13 [3–5,41]. Conclusions drawn from the replacement of PCV-7 with PCV-13 ranged from borderline cost-effective (England) [41] to cost-saving (USA, Germany, Greece, and The Netherlands) [3–5]. Although there are lots of differences between our study and theirs, we share the same determination in evaluating the replacement of PCV-7 to PCV-13,

as it is highly relevant to countries where PCV-7 has been offered under the national immunisation programme.

Our analysis is simple and straightforward based on the limited knowledge of epidemiology, and the assumption we made on efficacy or effectiveness of PCV-7 and PCV-13 may suggest an overestimation or underestimation of the results. However, evidences adopted are the best available ones to date, and assumptions made are the most conservative under the current uncertainty. The main limitations of our study are as follows: First, clinical evidences which show the effectiveness of vaccination in reducing annual incidence rates of the diseases in our model are adopted from studies carried out in other countries, since no similar study has been done in Japan. There should be differences in ethnicity as well as in the health system between those countries and Japan. Second, annual incidence rate of hospitalised pneumonia used in this study is based on a study done in only one prefecture because of the unavailability of national surveillance data, and such data would have a bias. Third, we did not include the benefits of vaccination in preventing antibiotic resistance in our model. Including this benefit would bring more cost-effective results given that the serotypes identified as penicillin resistant and covered by PCV-7 is above 80% in Japan [8,29].

5. Conclusion

Our study finds that if PCV-13 had additional protection against AOM compared to PCV-7 and cost per PCV-13 shot is 1.7 times less than that of PCV-7, 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 1.2 times less than that of PCV-7, replacing PCV-7 with PCV-13 will save money and gain more QALYs. However, if PCV-13 had no additional protection against AOM, the replacement can only be acceptable if cost per PCV-13 shot is 1.1 times less than of that of PCV-7. Due caution is needed in transferring these findings from our Japanese model to other health system, even so, replacing PCV-7 with PCV-13 to protect the birth cohort could be economically acceptable in developed countries.

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Original Article

Early therapy with neuraminidase inhibitors for influenza A (H1N1) pdm 2009 infection

Takehisa Yamamoto,[1] Mai Ihashi,[1] Yoshimi Mizoguchi,[1] Hiroshi Kaneno,[1] Katsusuke Yamamoto,[1] Yutaka Inoue,[2] Tetsuo Kase,[3] Nobuhiko Okabe[4] and Tsunesuke Shimotsuji[1]

Departments of [1]Pediatrics and [2]Radiology, Minoh City Hospital, [3]Osaka Prefectural Institute of Public Health, Osaka and [4]Kawasaki City Institute for Public Health, Kawasaki, Japan

Abstract ***Background***: Neuraminidase inhibitors have been reported to decrease mortality in patients infected with influenza A (H1N1) pdm 2009 (H1N1 pdm09), but it is not clear whether they are effective againstH1N1pdm09 in apparently healthy children.

Methods: The effect of early treatment with neuraminidase inhibitors on 70 otherwise healthy children with possible H1N1 pdm09 (pH1N1pdm09) infection was investigated. The children were simultaneously treated with a neuraminidase inhibitor (oseltamivir or zanamivir) and maoto, a Japanese traditional herbal medicine, which had been reported to be effective against seasonal influenza. Clinical severity was assessed using patient history, namely the worst values for clinical vital signs and laboratory data on admission. After refining these parameters with univariate, decision tree and multiple regression analysis, mean covariance structure equation analysis was used to investigate the association of estimated clinical severity to the selected parameters.

Results: Total path analysis using a Bayesian method indicated that the estimated clinical severity of pH1N1pdm09 was positively associated with maximum body temperature, pulse rate, respiration rate, duration necessary for defervescence, admission duration and log urinary β2-microglobulin/creatinine level, and negatively associated with age and the presence and duration of treatment with the neuraminidase inhibitor in the outpatient clinic.

Conclusions: This study provides the first clinical evidence that early treatment with neuraminidase inhibitors in outpatient clinic decreased the estimated clinical severity of pH1N1pdm09 in apparently otherwise healthy pediatric inpatients.

Key words early therapy, estimated clinical severity, influenza A (H1N1) pdm 2009, mean covariance structure equation analysis, neuraminidase inhibitor.

Neuraminidase inhibitors decrease mortality rate and the frequency of intensive care unit (ICU) admissions among patients with severe influenza A (H1N1) pdm 2009 (H1N1 pdm09) infection.[1,2] Also, early treatment with neuraminidase inhibitors was reported to reduce both the ICU and hospital stays of adults, compared to late treatment,[3] but it was recently reported that treatment with neuraminidase on admission did not affect the hospital stay of infants infected with H1N1 pdm.[4] In addition, there have been no reports on whether early treatment with neuraminidase inhibitors in the outpatient clinic affects clinical severity in pediatric patients hospitalized with mild bronchitis or pneumonia caused by H1N1 pdm09. In Japan, neuraminidase inhibitors have been commonly used to treat seasonal influenza[5] and were also prescribed to children suffering from H1N1 pdm09 from 2009 to 2010.[6] Thus, we thought it possible to evaluate the effect of early treatment with neuraminidase inhibitors in outpatient clinic on clinical severity in pediatric patients admitted to hospital with possible H1N1 pdm09 (pH1N1pdm09) infection. The definition of pH1N1pdm09 includes influenza A determined on rapid antigen test (Espline, Fujirebio, Japan) and confirmed as H1N1pdm09 on reverse transcription–polymerase chain reaction (RT-PCR), because almost all influenza A cases isolated in Osaka were confirmed as H1N1pdm on RT-PCR during the time of the present study.

The clinical severity of H1N1pdm is thought to be influenced by past history, clinical signs and symptoms and laboratory data on admission. To explore the precise relation between clinical severity and the effect of early treatment with neuraminidase inhibitors, we used mean covariance structure equation analysis.[7] This method combines factor and path analyses and enables testing of the model hypothesis simultaneously. Thus, it is thought to be a multivariate analysis by which it is possible to evaluate multiple relationships among clinical parameters at the same time.[8] Recently, Beran and Violato reported that this type of

Correspondence: Takehisa Yamamoto, MD, Department of Pediatrics, Minoh City Hospital, 5-7-1 Kayano, Minoh City, Osaka 562-8562, Japan. Email: t.yamamoto@minoh-hp.jp

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analysis was equal or superior to conventional regression analysis in terms of statistical power.[9] In fact, because of its usefulness, medical science has increasingly adopted it.[10,11]Thus, we used this method to assess the clinical severity of pH1N1pdm09 in pediatric patients hospitalized with bronchitis or pneumonia in relation to treatment with neuraminidase inhibitors in outpatient clinic. This study provides the first clinical evidence that early treatment with neuraminidase inhibitors in the outpatient clinic decreases the estimated clinical severity of pH1N1pdm09 in child inpatients.

Methods

Subjects

A total of 71 Japanese pediatric patients (46 boys, 25 girls) were admitted to Minoh City Hospital between September 2009 and January 2010. They were suffering from bronchitis or mild pneumonia caused by a pH1N1pdm infection. All of them were treated with anti-influenza medication using a neuraminidase inhibitor (oseltamivir or zanamivir) and maoto.[12] Maoto is a Japanese traditional herbal medicine reported to be effective against seasonal influenza infection.[12]We excluded a case of selective IgG3 deficiency.[13] Admission criteria were low pulse oxygen saturation (SpO_2) <95% with a cough and/or tachypnea accompanying a general state of not doing well. Sixty-six of 70 patients tested positive for influenza A with a rapid antigen test kit. Eighteen of 70 patients tested positive for H1N1pdm09 on RT-PCR, 14 of 18 were positive with both a rapid test kit and RT-PCR, and four of 18 were positive only on RT-PCR. The presence of pneumonia was confirmed by chest roentgenogram on admission. All the patients were healthy except for a present and/or past history of asthma (24 out of 70) or other allergic diseases such as allergic rhinitis and atopic dermatitis in addition to asthma (25 out of 70) and 16 patients had both categories of past history. The study protocol was approved by the review board of the Minoh City Hospital Clinical Ethics Committee. We obtained informed consent from the parents/guardians of all participants in the verbal form, which was written in the patient's records. National registration of this protocol by written consent was not needed because maoto is a Japanese traditional herbal medicine reported to be effective against seasonal influenza infections and approved for use as an anti-influenza medication by the Ministry of Health in Japan.

We also treated patients with pH1N1pdm09 using oral or i.v. antibiotics (47 of 70), when laboratory data on admission and thereafter indicated increased serum C-reactive protein (CRP) level (>5 mg/dL) or increased white blood cell (WBC) count (>15 000/μL). If the patients had an asthma attack, we used a β2-agonist with (5 of 70) and without (27 of 70) a continuous nebulized formulation (Inspiron; Japan Medicalnext, Osaka, Japan) and also i.v. methylprednisolone 3–4.5 mg/kg per day (22 out of 70), depending on patient clinical condition. In a few cases, we used gammaglobulin infusion; 1 g/kg per day once (3 of 70) on the occasion of a possible hypercytokinemia, which was judged from the elevation of urinary b2microglobulin/creatinine (u-b2MG/Cr).[14]

Clinical data and laboratory tests

The maximum temperature, respiration rate and pulse rate within 48 h of admission were taken from electronic patient records (Software service, Osaka, Japan). Serum and urinary constituents were collected within 48 h of admission and measured with standard techniques used at Minoh City Hospital. u-b2MG/Cr was assayed in house by a latex agglutination method.

Statistical analysis

Data are shown as mean and 95% confidence interval (95%CI). Significant differences for univariate analysis were assessed using one-way analysis of variance (ANOVA), Student's *t*-test or Fisher's exact test. Correlations between the parameters were evaluated using Spearman's correlation analysis (JMP 8.02; SAS, Cary, NC, USA).

First, we selected parameters for mean covariance structure analysis from among maximum body temperature, respiration rate, heart rate, duration necessary for defervescence below 37.5°C and admission duration, by multiple regression analysis (backward method; Dr SPSSII; IBM-SPSS, Chicago, IL, USA). The criterion for selection was $P < 0.1$. Next, we performed decision tree analysis (partition) using JMP 8.02 to reduce the number of parameters selected on univariate analysis ($P < 0.1$). Then, we selected parameters related to maximum body temperature, respiration rate, pulse rate, and duration necessary for defervescence below 37.5°C, using multiple regression analysis (backward method; $P < 0.1$; Table 1). Finally, we used mean covariance structure equation analysis using AMOS 16.0 (IBM-SPSS). We placed a corresponding path according to the results of the two multiple regression analyses. We also placed all the partial correlations between all the corresponding pairs of parameters when they had a significant correlation according to $P \leq 0.05$ (Table 2). We then added a new latent parameter: the estimated clinical severity of pH1N1pdm09, to evaluate its association with the selected parameters. Last, we set the error for covariance of the maximum pulse rate (e2) at 0 to avoid a problem of improper solutions in the mean covariance structure analysis. This method combined factor and path analysis, and enabled us to test the model hypothesis simultaneously. In detail, measured (clinical data) and latent (estimated clinical severity) variables were factored into the model and various models were tested for goodness of fit, which is usually judged from lowest root mean square error of approximation (RMSEA) <0.05, Akaike information criterion (AIC) score as low as possible and a comparative fit index (CFI) score as near to 1 as possible.[15] To evaluate the statistical significance in the total path analysis, we used the Bayesian method in AMOS 16.0 to calculate the mean and standard error. We used a coefficient of 3.29 as the standard error in order to evaluate *P*. To solve the problem of multiplicity, $P < 0.011$ was regarded as statistically significant according to the false discovery rate.[16]

Results

Baseline characteristics

In the outpatient clinic, the incidence of high fever (>38°C), cough and nausea or vomiting was 90%, 84% and 29%, respectively

Table 1 Selection of indicators of estimated clinical severity of H1N1 pdm09

Parameters	Univariate analysis		Decision tree analysis	Multiple regression analysis	
Dependent	Independent	*P*	Sum of squares	Beta	*P*
Max BT					
Outpatient clinic					
	Gender	0.070	4.4		
	SpO2	0.031	18		
On admission					
	Total chest Xp score	0.049	4.7	0.27	0.019
	Inhalation Tx with β2-agonist without Inspiron	0.029	2.1		
	Methylprednisolone i.v. Tx	0.0033	2.4		
	Duration of NI Tx in the outpatient clinic	0.0060	19	−0.42	<0.001
Max RR					
Outpatient clinic					
	Age	<0.0001	2 584	−0.48	<0.001
On admission					
	WBC	0.028	521		
	Platelet	0.0001	480		
	CK	0.0035	506	0.17	0.097
	U-log(b2MG/Cr)	0.0011	117		
	Pneumonia score	0.028	26		
	Inhalation Tx with β2-agonist without Inspiron	<0.0001	833	0.46	<0.001
	Methylprednisolone i.v. Tx	0.0003	1 342		
Max PR					
Outpatient clinic					
	Age	<0.0001	13 099	−0.51	<0.001
On admission					
	WBC	<0.0001	3 484	0.32	<0.001
	CRP	0.098	449		
	U-log(b2MG/Cr)	0.0008	4 115	0.17	0.041
	Atelectasis(+)	0.016	1 041		
	Total chest Xp score	0.031	390		
	Duration from the onset of flu	0.0036	785		
	Inhalation Tx with β2-agonist without Inspiron	0.011	133	0.29	<0.001
	Presence of NI Tx in the outpatient clinic	0.0041	908	−0.17	0.033
Duration necessary for defervescence					
Outpatient clinic					
	SpO2	0.076	707		
On admission					
	U-log(b2MG/Cr)	<0.0001	5 526	0.43	0.001
	Total Xp score	0.033	2 624		
	Duration from the onset of flu	0.0088	435		
	Duration of NI Tx in the outpatient clinic	0.0072	864		
Admission duration					
On admission					
	WBC	0.042	33		
	U-log(b2MG/Cr)	0.016	8.1		
	Total chest Xp score	<0.0001	2.6		
	Duration from the onset of flu	0.022	6.9		
	Inhalation Tx with β2-agonist without Inspiron	<0.0001	50	0.34	<0.001
	Antibiotics therapy	0.0051	33	0.34	<0.001
	Presence of NI Tx in the outpatient clinic	0.065	6.7	−0.25	0.008
	Duration of NI Tx in the outpatient clinic	0.080	52		
	Duration of NI Tx on admission	0.0027	132	0.48	<0.001

Corrected R^2 in decision tree analyses for maximum body temperature, respiration and pulse rate, duration necessary for defervescence and admission, by 5-fold cross-validation methods were 0.43, 0.63, 0.67, 0.24, and 0.56, respectively. Also, corrected R^2 in ANOVA were 0.21, 0.43, 0.66, 0.17, and 0.53, respectively. *P*-values of ANOVA for the above parameters were from <0.001 to 0.001. Only the data selected on decision tree analysis are given. BT, body temperature; CK, serum creatinine kinase; CRP, C-reactive protein; Inspiron, device for continuous nebulized formulation; Max, maximum; NI, neuraminidase inhibitors; PR, pulse rate; RR, respiration rate; Tx, therapy; U-log(b2MG/Cr), natural logarithm transformed urinary b2MG/creatinine; WBC, white blood cell; Xp, roentgenogram.

Table 2 Correlations between clinical parameters

	WBC	Antibiotic therapy	Inhalation Tx with β2-agonist without Inspiron	Presence of NI Tx in the outpatient clinic	Duration of NI Tx in the outpatient clinic	Duration of NI Tx on admission
Age	−0.36					
	**					
WBC		0.32				
		**				
CK		0.26	0.29			0.24
		*	*			*
U-log(b2MG/Cr)			0.28	−0.37	−0.46	
			*	**	***	
Total Xp score		0.46	0.33			
		***	**			
Presence of NI Tx in the outpatient clinic					0.92	0.30
					***	*
Duration of NI Tx in the outpatient clinic						0.32
						**

Upper numbers, ρ in Spearman's correlation analysis; lower symbols, $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. CK, serum creatinine kinase; NI, neuraminidase inhibitors; total chest Xp score, sum of pneumonia and atelectasis scores; Tx, therapy; u-log(b2MG/Cr), natural logarithm transformed urinary b2MG/creatinine; WBC, white blood cells.

(n = 70). The pulse oxygen saturation level was 96% (95%CI: 95–97%; n = 63). The duration from the onset of clinical signs and symptoms of influenza to the first visit for admission to a hospital was 2.8 days (95%CI: 2.4–3.2 days; n = 70). Thirty-seven percent of all patients (26/70) were pretreated with neuraminidase inhibitors before admission and the duration for taking neuraminidase inhibitors was 1.1 day (95%CI: 0.67–1.4 days; n = 70). Also, 7% of all patients (5/70) were pretreated with maoto before admission and the duration was 0.2 days (95%CI: 0–0.40 days; n = 70).

On admission, the mean patient age and duration of admission were 7.2 years old and 5.8 days with a 95%CI of 6.2–8.3 and 5.2–6.4, respectively (n = 70). Maximum temperature, respiration rate and pulse rate were 38.9°C (95%CI: 38.6–39.2°C), 40 breaths/min (95%CI: 37–42 breaths/min), and 139 beats/min (95%CI: 134–144 beats/min), respectively (n = 70). The duration necessary for defervescence under 37.5°C was 18.6 h (95%CI: 14.6–22.5 h; n = 70). Duration of neuraminidase inhibitor and maoto therapy were 6.0 days (95%CI: 5.4–6.5 days) and 5.1 day (95%CI: 4.8–5.4 days), respectively (n = 70).

Serum IgG, IgM and IgA were 927 mg/dL (95%CI: 832–1022 mg/dL), 112 mg/dL (95%CI: 99–125 mg/dL) and 115 mg/dL (95%CI: 86–143 mg/dL), respectively (n = 30). Blood analysis indicated a WBC count of 8321/μL (95%CI: 7189–9454/μL; n = 70), an increased percentage of granulocytes (68%; 95%CI: 62–74%; n = 46), and normal platelet counts of 24.2×10^4/μL (95%CI: 22.2–26.1 × 10^4/μL; n = 69). Fibrinogen degredation product (d-dimer) was increased to 1.5 μg/dL (95%CI: 1.0–2.0 μg/dL; n = 23). Biochemical markers showed increased CRP (2.2 mg/dL, 95%CI: 1.4–3.0 μg/dL; n = 70), normal lactate dehydrogenase of 267 IU/L (95%CI: 252–281 IU/L; n = 67) and increased serum creatinine kinase: 135 IU/L (95%CI: 110–146 IU/L; n = 67). In contrast, the mean natural logarithm transformed u-b2MG/Cr was elevated to 7.4 (95%CI: 7.0–7.8; n = 65).

The incidence of atelectasis and of pneumonia were 20% (14/70) and 83% (58/70), respectively. Severity was evaluated by summing the number of atelectasis in both lungs (0.23, 95%CI: 0.11–0.34; n = 70), and the pneumonia score (based on the area of pneumonia: 0, no pneumonia; 1 and 2, pneumonia in an area covering less and more than half of the unilateral lung, respectively: 2.3, 95%CI: 1.9–2.6; n = 70). The severity of chest roentgenogram (Xp) score was finally 2.5 (95%CI: 2.1–2.9; n = 70).

Multiple regression analysis was used to investigate the association among maximum body temperature, respiration rate, pulse rate, duration necessary for defervescence and admission duration.

The following parameters were selected for each item, respectively. Duration necessary for defervescence ($P < 0.001$) and maximum pulse rate ($P = 0.001$) for maximum body temperature; maximum pulse rate ($P < 0.001$) and admission duration ($P = 0.013$) for maximum respiration rate; maximum respiration rate ($P < 0.001$) and body temperature ($P = 0.041$) for maximum pulse rate; maximum body temperature ($P < 0.001$) for duration necessary for defervescence; and maximum respiration rate ($P = 0.004$) and body temperature ($P = 0.034$) for admission duration.

Univariate followed by decision tree and multiple regression analysis were done for selecting parameters related to maximum body temperature, respiration rate, pulse rate, duration necessary for defervescence and admission duration, respectively. The results are listed in Table 1.

Correlations between the parameters selected in Table 1

Results are shown in Table 2. Thirteen pairs of correlations were identified as statistically significant ($P < 0.05$).

Mean covariance structure equation analysis

The final model (Fig. 1) achieved a best fit of CFI = 0.982, RMSEA = 0.042 and AIC = 223. In path analysis by mean

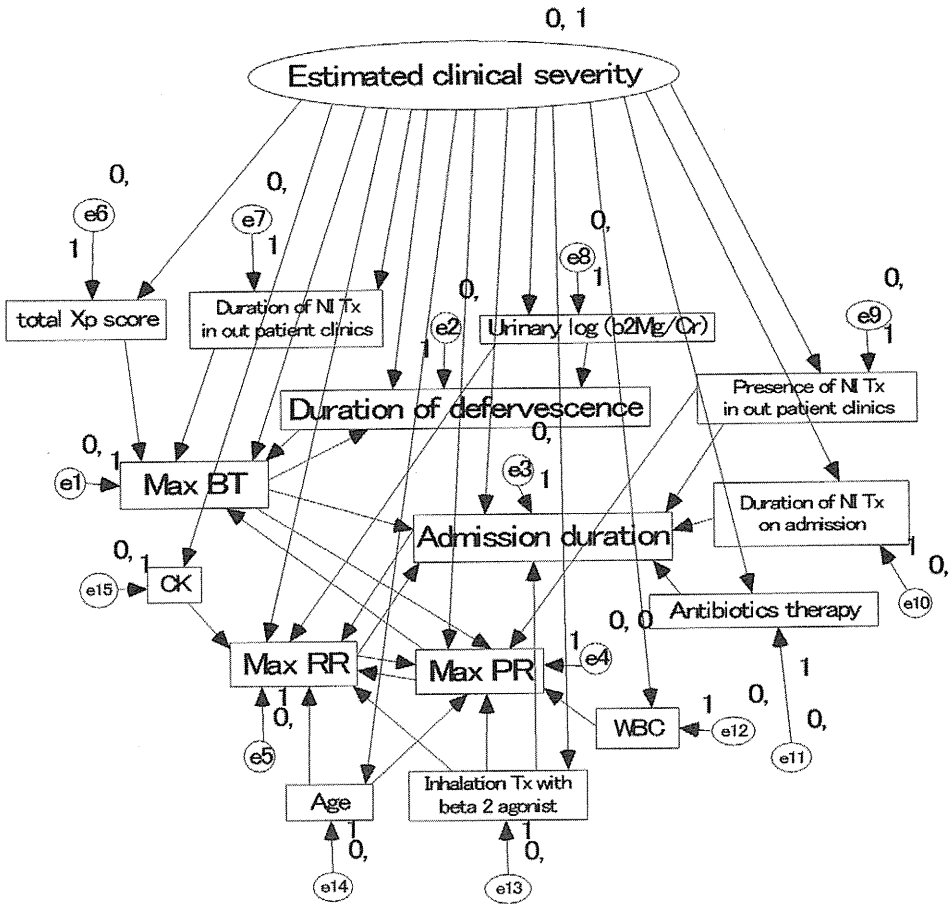

Fig. 1 Path analysis for the clinical severity of H1N1 pdm influenza. The covariance of maximal pulse rate error (e4) was fixed to 0 to have an established statistical model. To achieve simplicity, correlations between the following two errors for the corresponding parameters were not shown; e6 to e11, e13; e7 to e8, e9, e10; e8 to e9, e13; e9 to e10; e10 to e15; e11 to e12, e15; e12 to e14 and e13 to e15. Then the stable index was 0.42. The final model achieved the best satisfactory fit (CFI = 0.982, RMSEA = 0.042, AIC = 223). BT, body temperature; CK, serum creatinine kinase; Inspiron, a device for continuous nebulized formulation; Max, maximum; NI, neuraminidase inhibitors; PR, pulse rate; RR, respiration rate; Tx, therapy; U-log(b2MG/Cr), natural logarithm transformed urinary b2MG/creatinine; WBC, white blood cell; Xp, roentgenogram.

covariance structure equation analysis, we are able to evaluate three kinds of effects between the two parameters: direct (e.g. from A to C), indirect (e.g. from A to C via B), and total effects (i.e. the sum of the direct and indirect effects). In clinical medicine, total effects seem to be important because they include comprehensive effects.

Direct path analysis (Table 3) identified maximum body temperature as being significantly associated with estimated clinical severity ($P < 0.001$). For hospital stay, the duration of treatment with neuraminidase inhibitors given on admission, antibiotic therapy and inhalation therapy with a b2-agonist without Inspiron were all shown to be significant adverse factors ($P < 0.001$), while the presence of neuraminidase inhibitors given in the outpatient clinic ($P < 0.001$) was a significant ameliorating factor. Total path analysis (Fig. 2) by the Bayesian method identified duration and presence of neuraminidase inhibitor therapy in the outpatient clinic as being significantly associated with a marked reduction in the clinical severity of pH1N1pdm09, with mean path coefficients of −0.44 and −0.32, respectively ($P < 0.001$). Also, maximum body temperature, pulse rate, duration necessary for defervescence, respiration rate, admission duration and urinary log (b2MG/Cr) had significant positive associations with the estimated clinical severity of pH1N1pdm09, with a mean path coefficient of 0.86, 0.78, 0.64, 0.56, 0.47 and 0.46, respectively ($P < 0.001$).

Discussion

This study provides the first clinical evidence, obtained with mean covariance structure equation analysis, that early treatment with neuraminidase inhibitors in the outpatient clinic decreases the estimated clinical severity of pH1N1pdm in apparently otherwise healthy pediatric patients.

The mortality rate for H1N1 pdm09 in Japan is remarkably low compared to other advanced countries.[1] Also, Sugaya reported the widespread use of neuraminidase inhibitors during the pandemic caused by H1N1 pdm09 in Japan.[6] Given that the early treatment with neuraminidase inhibitors decreased the viral load of H1N1 pdm09,[17] and the prescription of neuraminidase

Table 3 Results of direct path analysis

Origin	End	Standardized path coefficient	*P*
Clinical severity	Max BT	0.78	<0.001
Clinical severity	Inhalation of b2-agonist without Inspiron	0.28	0.024
Clinical severity	Duration of NI Tx in outpatient clinic	−0.37	0.033
Duration of NI Tx on admission	Admission duration	0.49	<0.001
Antibiotics therapy	Admission duration	0.44	<0.001
Inhalation of b2 agonist without Inspiron	Admission duration	0.43	<0.001
Presence of NI Tx in outpatient clinic	Admission duration	−0.32	<0.001
WBC	Max PR	0.29	<0.001
Age	Max PR	−0.42	<0.001
Admission duration	Max RR	0.39	0.006
Age	Max RR	−0.43	0.005

Only $P < 0.05$ are shown. The criterion for statistical significance is $P < 0.011$. BT, body temperature; NI, neuraminidase inhibitors; PR, pulse rate; RR, respiration rate; Tx, therapy; u-log(b2MG/Cr), natural logarithm transformed urinary b2MG/creatinine; WBC, white blood cells.

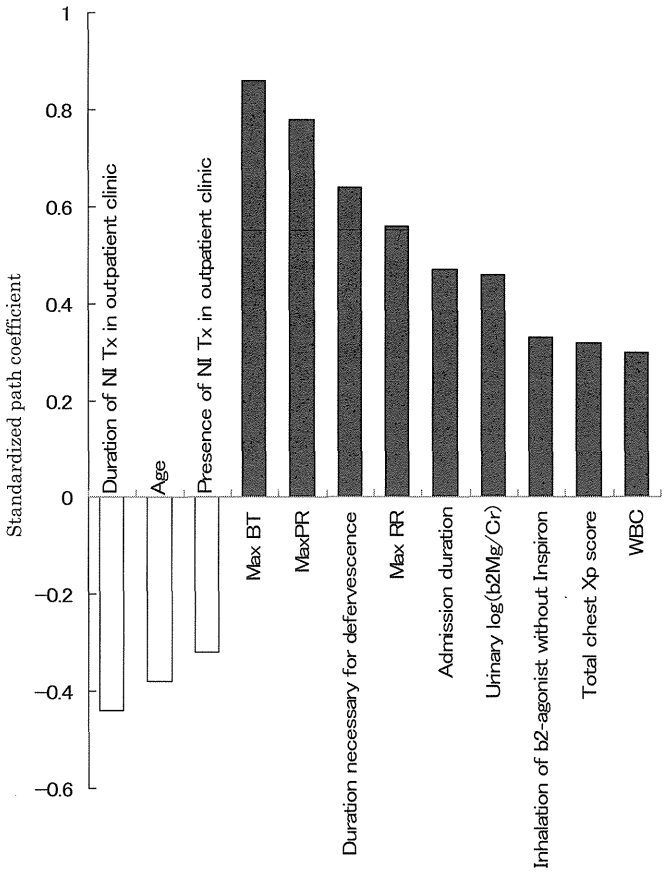

Fig. 2 Effects of estimated clinical severity on the measured parameters by total path analysis. Only absolute values for standardized path coefficients ≥0.30 are shown. *P* for all the parameters was <0.001. BT, body temperature; Inspiron, device for continuous nebulized formulation; NI, neuraminidase inhibitors; PR, pulse rate; RR, respiration rate; Tx, therapy; u-log(b2MG/Cr), natural logarithm transformed urinary b2MG/creatinine; WBC, white blood cells; Xp, roentgenogram.

inhibitors prevented hospitalization caused by H1N1 pdm09,[18] the present data on the effectiveness of early therapy with neuraminidase inhibitors in reducing the clinical severity of pH1N1pdm09 in apparently otherwise healthy children are convincing. It was recently reported that early initiation of neuraminidase inhibitors during the 2009–2010 H1N1pdm09 epidemic reduced the likelihood of severe outcomes compared with late or no treatment.[19]

In this paper, we used mean covariance structure equation analysis to clarify the association of clinical severity of pH1N1pdm09 with several clinical parameters. This analysis was recently proposed as a powerful analysis equal or superior to conventional linear regression analysis.[8] In particular, this method enabled us to perform statistical analysis including non-measured parameters (latent variables). In the present case, we defined a latent variable as estimated clinical severity in patients with pH1N1pdm09. We believe that the estimated clinical severity was valid, because it had a significantly positive association with maximum body temperature ($P < 0.001$) in direct path analysis.

Direct path analysis also indicated that antibiotic therapy was positively associated with admission duration. This suggested the possibility of bacterial co-infection in patients with pH1N1pdm09 in the present study, which was compatible with the reports of pneumococcal and *Haemophilus influenzae* type b co-infection in H1N1 pdm09.[20,21] We could not, however, confirm bacterial co-infection, because we could not isolated these bacteria on blood or deep nasal swab cultures.

In addition, the presence of neuraminidase inhibitor therapy in the outpatient clinic was proved to negatively associate with admission duration on direct path analysis. In contrast, there were two reports indicating that neuraminidase inhibitors were not associated with length of hospital stay in infants[4,22] Thus, the question might arise as to why we were able to show that neuraminidase given in the outpatient clinic significantly shortened admission duration on direct path analysis. The discharge criteria at Minoh City Hospital were as follows: continuous duration of

defervescence for no more than 1 day, absence of tachypnea and asthma attacks and improved general condition in terms of appetite and normal daily activities. Given that these criteria seem to be similar to those of other hospitals, the reason why we found a difference in admission duration might be the timing of neuraminidase treatment. This speculation is compatible with a report that time from onset of symptoms to oseltamivir treatment was associated with prolonged hospital stay in adults.[23] An alternative reason could be the increased statistical power of the mean covariance structure equation analysis, in that R^2 for admission duration in the present study was 0.50.

Total path analysis, which is thought to be important in clinical medicine, identified estimated clinical severity as significantly associated with maximum body temperature, pulse rate, duration necessary for defervescence, maximum respiration rate and total Xp score. These results were consistent with daily clinical experience. Moreover, the significant positive association of estimated clinical severity to therapy with a b2-agonist without Inspiron and to urinary log (b2MG/Cr) level is thought to be linked to asthma attack[24] and to elevated serum cytokine level,[25] which were reported to be encountered in H1N1pdm09 infection, respectively.

There were some limitations in this report. First, not all of the present patients were confirmed as having H1N1 pdm09 on RT-PCR. This is a major problem in the present study. It might be partly resolved, however, because there was a pandemic of H1N1 pdm09 in Osaka, Japan during the time of this research. Second, we could investigate serum IgG subclass levels in only 44 patients (63% of the total) because of a financial problem due to the restricted application of Japanese insurance systems for laboratory investigations. This might affect the association of the estimated clinical severity with clinical parameters to some extent, because IgG3 deficiency was shown to be associated with delay in discharge from hospital.[12] This is probably not the case, however, because IgG subclass deficiency is reported to be rare,[26] although we could not exclude the possibility.

On the basis of these results, we thought it worthwhile to report the possible usefulness of early treatment with neuraminidase inhibitors for children in advance of any future pandemic of influenza.

In conclusion, mean covariance structure equation analysis showed that early neuraminidase inhibitor therapy in the outpatient clinic significantly reduced the estimated clinical severity among admitted pediatric patients with pneumonia or bronchitis caused by pH1N1pdm09.

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Immunogenicity and Safety after Booster Vaccination of Diphtheria, Tetanus, and Acellular Pertussis in Young Adults: an Open Randomized Controlled Trial in Japan

Megumi Hara,[a] Kenji Okada,[b] Yuko Yamaguchi,[c] Shingo Uno,[c] Yasuko Otsuka,[a] Chisato Shimanoe,[a] Hinako Nanri,[a] Mikako Horita,[a] Iwata Ozaki,[d] Yuichiro Nishida,[a] Keitaro Tanaka[a]

Department of Preventive Medicine, Faculty of Medicine, Saga University, Saga City, Japan[a]; Department of Pediatrics, Fukuoka Dental College, Fukuoka City, Fukuoka, Japan[b]; The Chemo-Sero-Therapeutic Research Institute (Kaketsuken), Kumamoto City, Kumamoto, Japan[c]; Health Care Center, Saga University, Saga City, Saga, Japan[d]

The recent increase of pertussis in young adults in Japan is hypothesized to be due in part to waning protection from the acellular pertussis vaccine. While a booster immunization may prevent an epidemic of pertussis among these young adults, little is known about the safety and immunogenicity of such a booster with the diphtheria, tetanus, and acellular pertussis vaccine (DTaP), which is currently available in Japan. One hundred and eleven medical students with a mean age of 19.4 years were randomly divided into 2 groups of 55 and 56 subjects and received, respectively, 0.2 or 0.5 ml of DTaP. Immunogenicity was assessed by performing the immunoassay using serum, and the geometric mean concentration (GMC), GMC ratio (GMCR), seropositive rate, and booster response rate were calculated. Adverse reactions and adverse events were monitored for 7 days after vaccination. After booster vaccination in the two groups, significant increases were found in the antibodies against pertussis toxin, filamentous hemagglutinin, diphtheria toxoid, and tetanus toxoid, and the booster response rates for all subjects reached 100%. The GMCs and GMCRs against all antigens were significantly higher in the 0.5-ml group than in the 0.2-ml group. No serious adverse events were observed. Frequencies of local reactions were similar in the 2 groups, although the frequency of severe local swelling was significantly higher in the 0.5-ml group. These data support the acceptability of booster immunization using both 0.2 and 0.5 ml of DTaP for young adults for controlling pertussis. (This study was registered at UMIN-CTR under registration number UMIN000010672.)

During the last few decades, the number of reported pertussis cases has increased in developed countries, despite high vaccination coverage (1). This resurgence of reported pertussis has been hypothesized to be due to several reasons, including increased awareness of pertussis; use of PCR assay for diagnosis; failure of the diphtheria, tetanus, and acellular pertussis vaccine (DTaP); and genetic changes in circulating strains of *Bordetella pertussis* (2, 3). DTaP does not confer lifelong immunity, and it has been reported to last for 4 to 12 years after infant immunization (4). A recent study demonstrated that after the fifth dose of DTaP, protection against pertussis waned during the following 5 years, and the risk of pertussis increased by an average of 42% per year (5).

The prevalence of pertussis in Japan was estimated to be 2.4 (95% confidence interval, 1.6 to 3.3) per 100,000 population in 2007 (see the National Institute of Infectious Diseases fact sheet for pertussis vaccine [in Japanese] at http://www.mhlw.go.jp/stf/shingi/2r9852000000bx23-att/2r9852000000byfg.pdf), while the prevalence in the United States was reported to be 9.0 per 100,000 population in 2010 (3). It is difficult to compare these values, because of differences of diagnostic methods applied and case definitions for surveillance. However, the proportion of adults among recently reported pertussis cases has been increasing in Japan (see the National Institute of Infectious Diseases fact sheet), even though underreporting of adult cases was suspected due to the fact that pertussis cases were primarily reported from pediatric clinics. In Japan, children receive 4 doses of the DTaP vaccine, with 3 primary doses and a single booster dose at ages 3, 4, 5, and 18 to 23 months. Thus, a decreased protective effect of the vaccine may contribute to the increasing frequency of pertussis in the last decade on college campuses and in high schools and offices in Japan (6–10). Pertussis prevention among young adults is important because unrecognized adult pertussis is the major source of pertussis in young infants, in whom the disease can be severe and fatal (2).

The tetanus, reduced antigen content diphtheria, and acellular pertussis vaccine (Tdap) is used as a booster vaccination worldwide for adults, and its effects in adolescents and adults, as well as in specific risk groups, such as pregnant women and their newborns, health care workers, and older adults, have been reported (11–13). Since Tdap has not yet been licensed in Japan, DTaP may be available for booster immunization in the interim. Safe and effective booster immunization using DTaP in adolescents has been confirmed (14); however, little is known about the immunogenicity and safety of the DTaP vaccine in young adults. In this study, we examined the immunogenicity and safety of 0.2 and 0.5 ml of DTaP in young adults in Japan.

MATERIALS AND METHODS

Study subjects and design. The participants were recruited at the Saga University, located in southern Japan, where an outbreak of pertussis had

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Address correspondence to Megumi Hara, harameg@cc.saga-u.ac.jp.

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occurred among medical students during April and May in 2010. After the outbreak, we used an enzyme-linked immunosorbent assay (ELISA) at a commercial laboratory (SRL, Tokyo) to examine antibodies against pertussis toxin (PT) in all 548 students during July and August 2010. We found that the levels of antibodies against PT among 258 students (47%) were <10 ELISA units (EU)/ml, and those among 24 students (4%) were ≥100 EU/ml. We announced the participation of these students in this study during August 2011. Students were excluded from participation if their antibody levels against PT were ≥100 EU/ml in 2010; if they had any history of diphtheria, tetanus, and pertussis; if they had received any other drug or vaccine within 30 days of entry; if they had a history of allergic reactions to any vaccine component; or if they had received immunoglobulins or any blood products. Subjects were also excluded if they had acute disease or febrile illness at the time of vaccination. Overall, 111 students aged 19 to 20 years participated in this study. They had undergone primary vaccination with the DTaP vaccine in Japan during childhood. The immunization histories of 83 subjects were confirmed by checking their immunization records, whereas those of the remaining 28 subjects could not be verified because they did not submit their records.

This study (registered at UMIN-CTR under registration number UMIN000010672) was an open randomized controlled trial performed using blocked randomization for gender and prevaccination antibody levels against PT. The study subjects were stratified according to seropositivity for PT (PT-IgG, ≥10 EU/ml) or nonseropositivity for PT (PT-IgG, <10 EU/ml) in the previous year, and according to gender. Then, the subjects within each group were randomly assigned to receive 0.2 or 0.5 ml of DTaP vaccine. Serum samples were obtained before immunization and 1 month after immunization. All serum specimens were stored at −80°C until assayed. All subjects were included in the safety and immunogenicity analysis.

The study was conducted in accordance with good clinical practice guidelines and the principles of the Declaration of Helsinki and Japanese regulatory requirements. The study protocol was approved by the Institutional Review Board of the Saga University Faculty of Medicine (approval number 23-14, 2011). The nature and possible consequences of the study were discussed at length with all subjects, and written informed consent for participation was obtained from all.

Vaccines. The single doses of 0.2 and 0.5 ml of adsorbed diphtheria-purified pertussis-tetanus combined vaccine (DTaP vaccine, lot number 42A; Kaketsuken) contained 3.2 and 8 μg of PT, 12.8 and 32 μg of filamentous hemagglutinin (FHA), ≤6.7 and ≤16.7 Lf of diphtheria toxoid (D), and ≤2.7 and ≤6.7 Lf of tetanus toxoid (T), respectively. This DTaP also contained 0.004 mg/ml of thimerosal, <3 mg/ml of aluminum hydroxide, and <1 mg/ml of formalin. The vaccine was administered subcutaneously in the lateral upper arm by using a 27-gauge needle of length 25 mm.

Assessment of safety. All subjects were carefully observed for anaphylactic shock for at least 30 min after vaccination. To assess for adverse reactions occurring during the initial 24 h and the 7 days immediately following vaccination, the subjects were asked to perform daily self-assessments and record their body temperature at the axillary fossa and their adverse reactions based on a standard health care diary. Adverse reactions occurring within 24 h included the following: fever (temperature of ≥38.0°C), eye hyperemia, facial edema, cough, dyspnea, dysphasia, hoarseness, and sore throat. After 24 h, the adverse local symptoms consisted of erythema, swelling, pain, warmth, and pruritus, whereas systemic reactions included fever (temperature of ≥38.0°C), headache, fatigue, cough, and sore throat. The degrees of erythema and swelling in the local reactions were evaluated by the diameter of the swelling and were defined as follows: mild (<2.0 cm), moderate (2.0 to 4.9 cm), and severe (≥5.0 cm). The degrees of pain, warmth, and pruritus in local reactions were also categorized based on the patients' perceptions and defined as follows: mild, if the patient was aware of the reaction but not concerned; moderate, if the patient was anxious; and severe, if the patient required medication for pain and/or pruritus. All subjects were monitored for 6 months after vaccination to get the maximum information regarding unexpected serious adverse events.

Serological assays. Serum antibodies against PT, FHA, D, and T were concurrently measured by Kaketsuken. Antibodies to PT and FHA were measured using standard enzyme-linked immunosorbent assays (ELISA), and pertussis antibody titers were expressed as ELISA units (EU)/ml (15). Diphtheria antitoxin titers were examined using the micro cell culture method with Vero cells and expressed as international units (IU)/ml (16). Tetanus antitoxin titers were determined using a KPA kit (Kaketsuken, Japan) and expressed as international units (IU)/ml (17). Limits of quantification for each antibody against PT, FHA, D, and T were 1.0 EU/ml, 1.0 EU/ml, 0.005 IU/ml, and 0.005 IU/ml, respectively. Seropositive levels were defined as ≥10 EU/ml for antibodies against PT and FHA, ≥0.1 IU/ml for diphtheria antitoxin, and ≥0.01 IU/ml for tetanus antitoxin (14). A booster response for PT and FHA was defined as a postvaccination antibody concentration of ≥20 EU/ml with a prevaccination antibody concentration of <5.0 EU/ml, a postvaccination rise of at least 4 times the prevaccination antibody concentration in subjects with a prevaccination antibody concentration of 5.0 to 20 EU/ml, or at least twice the prevaccination antibody concentration in subjects with a prevaccination antibody concentration of ≥20 EU/ml. Booster responses for D and T were defined as a postvaccination antibody concentration of ≥0.4 IU/ml with a prevaccination antibody concentration of <0.1 IU/ml, or a postvaccination increase of at least 4 times the prevaccination antibody concentration with an initial concentration of ≥0.1 IU/ml (18).

Statistical analyses. The primary objective of the study was to demonstrate booster response rates of at least 80% for PT, FHA, D, and T in the two groups. The chi-square test or Fisher's exact test was used to compare the baseline characteristics or seropositivity rates and frequency of adverse reactions between the groups. The 95% confidence interval (CI) of seropositivity rates were calculated with the exact binomial distribution for proportions. Because distributions of antibodies were skewed, all antibody calculations were done using a log scale. The Wilcoxon rank-sum test was used to compare the GMC and GMC ratio (GMCR) between the 2 groups, while the Wilcoxon signed-rank test was used to determine the significance of the increase in antibodies after vaccination in each group. Hypothesis testing was conducted using two-sided tests, with an α value of 0.05 considered statistically significant. All statistical analyses were performed using the SAS software (version 9.1).

RESULTS

Baseline characteristics of the subjects. Baseline demographic characteristics of the 2 groups are shown in Table 1. There were no statistical differences between the 2 groups in the proportions of female subjects, age distributions, DTaP histories, current diseases, and allergy histories. Furthermore, the distributions of prevaccination antibodies and seropositivity rates before vaccination against PT, FHA, D, and T were similar in the 2 groups (Table 2).

Immunogenicity. After each DTaP vaccination, each group demonstrated significant increases in the GMC and GMCR of antibodies and seropositivity rates against PT, FHA, D, and T (Table 2). The immune responses were significantly lower in the 0.2-ml group than in the 0.5-ml group; however, both groups reached 100% seropositivity and booster response rates against all antigens. These results satisfied our primary objectives.

Safety. No serious adverse events occurred during the study period. Several adverse reactions occurred, but all were transient and occurred at similar frequencies in the 2 groups (Table 3). Within 24 h after vaccination, the most frequent symptoms were cough (3.6%), dysphasia (3.6%), and sore throat (3.6%). After 24 h, local reactions were frequently observed, with few systemic reactions. The onset of local reactions is summarized in

TABLE 1 Baseline characteristics of the study subjects

Characteristic	Values for subjects who received: 0.2 ml DTaP (n = 55)	0.5 ml DTaP (n = 56)	P^a
Female (n [%])	42 (76.4)	41 (73.2)	0.703
Age (mean ± SD) (yr)	19.4 ± 1.2	19.4 ± 0.8	0.97
DTP history (n [%])			
1 dose	1 (1.8)	0 (0)	0.617
3 doses	2 (3.9)	2 (3.9)	
4 doses	33 (63.5)	37 (72.6)	
Uncertain	16 (30.8)	12 (23.5)	
Disease (n [%])			
Asthma	1 (1.8)	0 (0)	0.496
Atopic dermatitis	2 (3.6)	2 (3.6)	1
Allergy history (n [%])			
Drugs	3 (5.4)	2 (3.6)	0.679
Foods	7 (12.7)	3 (5.4)	0.202

[a] P values were tested by the t, chi-square, or Fisher's exact test.

Table 4. Erythema, swelling, and pain were reported by more than half of the subjects. Most injection site reactions resolved within 3 days.

The severities of the local reactions are summarized in Table 5. The frequencies of severe local reactions tended to be higher in the 0.5-ml group than in the 0.2-ml group. Moreover, the frequency of local swelling was significantly higher in the 0.5-ml group than in the 0.2-ml group (relative risk [RR], 4.42; 95% CI, 1.00 to 19.54). However, none of these reactions affected the subjects' ordinary daily activities.

TABLE 3 Adverse reactions after vaccination

Time span and adverse reaction	No. (%) of subjects with adverse reaction to: 0.2 ml DTaP (n = 55)	0.5 ml DTaP (n = 56)	P^a
Within 24 h			
Fever (≥38.0°C)	0 (0)	1 (1.8)	1.000
Eye hyperemia	0 (0)	1 (1.8)	1.000
Face edema	0 (0)	1 (1.8)	1.000
Cough	2 (3.6)	2 (3.6)	1.000
Dyspnea	0 (0)	1 (1.8)	1.000
Dysphasia	0 (0)	2 (3.6)	0.495
Hoarseness	0 (0)	1 (1.8)	1.000
Sore throat	1 (1.8)	2 (3.6)	1.000
After 24 h			
Local reaction			
Erythema	39 (70.9)	33 (58.9)	0.186
Swelling	33 (60.0)	33 (58.9)	0.908
Pain	34 (61.8)	38 (67.9)	0.505
Hotness	23 (41.8)	27 (48.2)	0.444
Itching	27 (49.1)	25 (44.6)	0.639
Systemic reaction			
Fever (≥38.0°C)	0 (0)	1 (1.8)	1.000
Headache	0 (0)	2 (3.6)	0.495
Fatigue	3 (5.5)	2 (3.6)	0.679
Cough	1 (1.8)	2 (3.6)	1.000
Sore throat	2 (3.8)	1 (1.8)	0.618

[a] P values were tested by Fisher's exact test or chi-square test.

DISCUSSION

In this randomized clinical trial comparing 0.2 ml to 0.5 ml of DTaP vaccine in young adults, we showed that effective immunogenicity for PT, FHA, D, and T was achieved in both groups. All

TABLE 2 Antibody GMCs, seropositive levels, and booster responses after DTaP vaccinations[d]

Antibody	Dose (ml)	n	GMC concn (per ml [95% CI]) Prevaccination	Postvaccination	GMCR	P^b	No. (%) (95% CI) of subjects who were seropositive[e] Prevaccination	Postvaccination	No. (%) (95% CI) of subjects with a booster response[f]
Anti-PT	0.2	55	7.81 (5.31, 11.49) EU	90.90 (73.95, 111.74) EU	11.6	<0.0001	23 (42) (29, 56)	55 (100) (94, 100)	55 (100) (94, 100)
	0.5	56	6.83 (4.97, 9.40) EU	168.71 (141.93, 200.54) EU	24.7	<0.0001	21 (38) (25, 52)	56 (100) (94, 100)	56 (100) (94, 100)
P			0.6205[a]	<0.0001[a]	<0.001[a]		0.6434[c]	NC	NC
Anti-FHA	0.2	55	24.39 (16.76, 35.51) EU	213.36 (177.63, 256.26) EU	8.7	<0.0001	43 (78) (67, 88)	55 (100) (94, 100)	55 (100) (94, 100)
	0.5	56	20.71 (15.48, 27.72) EU	397.77 (333.80, 474.00) EU	19.2	<0.0001	40 (71) (58, 52)	56 (100) (94, 100)	56 (100) (94, 100)
P			0.4951[a]	<0.0001[a]	<0.001[a]		0.4148[c]	NC	NC
Anti-D	0.2	55	0.22 (0.16, 0.30) IU	4.29 (3.53, 5.21) IU	19.8	<0.0001	41 (75) (63, 86)	55 (100) (94, 100)	55 (100) (94, 100)
	0.5	56	0.21 (0.15, 0.30) IU	6.28 (4.86, 8.11) IU	30.1	<0.0001	39 (70) (56, 81)	56 (100) (94, 100)	56 (100) (94, 100)
P			0.9016[a]	0.0109[a]	0.01[a]		0.5666[c]	NC	NC
Anti-T	0.2	55	0.25 (0.18, 0.34) IU	1.46 (1.26, 1.69) IU	5.9	<0.0001	55 (100) (94, 100)	55 (100) (94, 100)	55 (100) (94, 100)
	0.5	56	0.27 (0.21, 0.35) IU	2.52 (2.14, 2.96) IU	9.4	<0.0001	56 (100) (94, 100)	56 (100) (94, 100)	56 (100) (94, 100)
P			0.9112[a]	<0.0001[a]	<0.001[a]		NC	NC	NC

[a] P values were calculated between groups by using the Wilcoxon rank-sum test with a log scale of antibodies.

[b] P values were calculated between prevaccination and postvaccination by using the Wilcoxon signed-rank test with a log scale of antibodies.

[c] P values were calculated between groups by using the chi-square test.

[d] CI, confidence interval; GMC, geometric mean concentration; GMCR, ratio of GMC of prevaccination to postvaccination; NC, not compared.

[e] Seropositive levels were defined as ≥10 EU/ml for antibodies against PT and FHA, ≥0.1 IU/ml for those against diphtheria toxoid, and ≥0.01 IU/ml for those against tetanus toxoid.

[f] Booster responses for PT and FHA were defined as postvaccination antibody concentrations of ≥20 EU/ml with a prevaccination antibody concentration of <5.0 EU/ml, a postvaccination rise of at least 4 times the prevaccination antibody concentration in subjects with a prevaccination antibody concentration of 5.0 to 20 EU/ml, or at least twice the prevaccination antibody concentration in subjects with a prevaccination antibody concentration of ≥20 EL U/ml. Booster responses for D and T were defined as a postvaccination antibody concentration of ≥0.4 IU/ml with a prevaccination antibody concentration of <0.1 IU/ml or a postvaccination increase of at least 4 times the prevaccination antibody concentration with an initial concentration of ≥0.1 IU/ml.

TABLE 4 Onset of local reactions

Local reaction	Vaccine dose (ml)	No. (%) of subjects with indicated local reaction: Day of onset 0	1	2	3	4	5	6	7	Total no. (%) with indicated reaction
Erythema	0.2	10 (18.2)	14 (25.5)	11 (20.0)	3 (5.5)	0	0	1 (1.8)	0	39 (70.9)
	0.5	9 (16.1)	14 (25.0)	8 (14.3)	1 (1.8)	0	1 (1.8)	0	0	33 (58.9)
Swelling	0.2	6 (10.9)	15 (27.3)	9 (16.4)	2 (3.6)	0	1 (1.8)	0	0	33 (60.0)
	0.5	8 (14.3)	15 (26.8)	8 (14.3)	2 (3.6)	0	0	0	0	33 (58.9)
Pain	0.2	11 (20.0)	14 (25.5)	7 (12.7)	2 (3.6)	0	0	0	0	34 (61.8)
	0.5	15 (26.8)	17 (30.4)	5 (8.9)	1 (1.8)	0	0	0	0	38 (67.9)
Hotness	0.2	7 (12.7)	10 (18.2)	4 (7.3)	2 (3.6)	0	0	0	0	23 (41.8)
	0.5	8 (14.3)	8 (14.3)	10 (17.9)	1 (1.8)	0	0	0	0	27 (48.2)
Itching	0.2	8 (14.5)	10 (18.2)	4 (7.3)	2 (3.6)	2 (3.6)	1 (1.8)	0	0	27 (49.1)
	0.5	6 (10.7)	12 (21.4)	6 (10.7)	0	1 (1.8)	0	0	0	25 (44.6)

subjects in the two groups demonstrated sufficient booster responses against all vaccine antigens. None of the severe adverse reactions observed required medications. The total number and onset of local and systemic reactions between the 2 groups were similar, although the frequency of severe local swelling was significantly higher in the 0.5-ml group ($P < 0.05$). Thus, both doses of DTaP may provide adequate booster immunization in young adults in Japan, where the Tdap vaccine has not yet been licensed.

Clinical trials evaluating the duration of protective immunity provided by 3 or 4 doses of DTaP against pertussis demonstrated that protective immunity was sustained for 5 to 6 years after immunization (19, 20). The immunization schedule of DTaP in Japan consists of 4 doses in young children during 4 to 23 months of age and 1 dose of DT during 11 and 12 years of age. Thus, in our study participants, 17 to 18 years had elapsed since their last immunization. In our study, the prevaccination antibody seropositivity rates against pertussis were around 40% for PT and 70 to 80% for FHA, which are lower than those reported in Japanese adolescents (14) and Finnish young adults (21). In the former study, seropositivity rates against pertussis were 52 to 59% for PT and 79 to 86% for FHA among the adolescents, who were immunized for pertussis about 10 years prior (14). In the latter study, seropositivity rates at 10 years after the fifth dose of Tdap were 61.3% for PT and 100% for FHA (21).

Since immunogenicity to vaccination is influenced by the prevaccination antibody level, vaccination history, doses of vaccine components, and laboratory where the antibodies were measured, it is difficult to compare immunogenicity between previous studies using Tdap and the present study. With regard to the prevaccination antibody level, we observed antibody levels against PT and FHA that were lower than those in previous studies (14, 21). Thus, immunogenicities in our subjects may have been lower than those of the previous studies if the components of the vaccine were the same. The 0.5 ml of DTaP vaccine (Kaketsuken) contains the same dose of PT and higher doses of FHA, D, and T than the Boostrix Tdap (13) and higher doses of PT, FHA, D, and T than

TABLE 5 Degrees of local reactions

Type of reaction[a]	Vaccine dose (ml)	No. (%) of subjects with: Absence of reaction	Reaction: Mild	Moderate	Severe	Risk ratio (95% CI)[b] for severe local reactions
Redness	0.2	16 (29)	5 (13)	26 (67)	8 (21)	1 (reference)
	0.5	23 (41)	5 (15)	17 (52)	11 (33)	1.35 (0.59–3.10)
Swelling	0.2	23 (42)	8 (24)	22 (67)	2 (6)	1 (reference)
	0.5	23 (41)	6 (18)	18 (55)	9 (27)	4.42 (1.00–19.54)
Pain	0.2	21 (38)	23 (68)	10 (29)	1 (3)	1 (reference)
	0.5	18 (32)	22 (58)	13 (34)	3 (8)	2.95 (0.32–27.47)
Warmth	0.2	32 (58)		20 (87)	3 (13)	1 (reference)
	0.5	29 (52)		21 (78)	6 (22)	1.96 (0.52–7.46)
Pruritus	0.2	28 (51)	14 (52)	12 (44)	1 (4)	1 (reference)
	0.5	31 (55)	6 (24)	16 (64)	3 (12)	2.95 (0.32–27.47)

[a] Degrees of redness and swelling: mild (<2.0 cm), moderate (2.0 to 4.9 cm), or severe (≥5.0 cm). Degrees of pain, warmth, and pruritus: mild (sensed, but not anxious about), moderate (anxious), or severe (needs medication).

[b] CI, confidence interval.

TABLE 6 Components of DTaP and Tdap vaccines

Vaccine	Manufacturer	PT (μg)	FHA (μg)	Pertactin (μg)	Fimbriae (μg)	D (Lf)	T (Lf)
DTaP, 0.5 ml	Kaketsuken	8	32			≤16.7	≤6.7
DTaP, 0.2 ml	Kaketsuken	3.2	12.8			≤6.7	≤2.7
Tdap, 0.5 ml (Boostrix)	GlaxoSmithKline Biological	8	8	2.5		2.5	5
Tdap, 0.5 ml (Adacel)	Sanofi Pasteur	2.5	5	3	5	2	5

the Adacel Tdap (12), which is used abroad (14) (Table 6). However, immunogenicities against PT in this study were sufficient despite 0.2 ml of DTaP, results which are very similar to those of previous reports (12–14, 21).

The most common local reactions reported in our study were erythema, swelling, and pain. The frequencies of these reactions were similar to those reported in Japanese adolescents aged 11 to 12 years (14) who had received 0.2 or 0.5 ml of DTaP. Compared to randomized clinical trials for Tdap, the frequency of injection site pain was similar, whereas there were more cases of erythema and swelling in our study. The frequencies of pain, swelling, and erythema in American adults who were vaccinated with the Adacel Tdap (12) were 65.7%, 21%, and 24.7%, respectively, and in those who had the Boostrix Tdap (13) were 61.0%, 21.1%, and 17.6%, respectively. These discrepancies in the frequencies of erythema and swelling between Japan and the United States may be due to the mode of injection. In the United States, Tdap is injected intramuscularly, while DTaP vaccine was administered subcutaneously in this study. The frequencies of other adverse reactions in this study did not differ from those in the clinical trials of Tdap (12, 13).

Several studies have examined the effects of vaccine antigen contents on immunogenicity and reactogenicity, and although immunogenicities differed between the studies, all demonstrated that local reactions can be reduced by decreasing the amount of antigen (14, 18, 22, 23). Knuf et al. assessed DTP vaccines with reduced amounts of antigens in the fourth dose in the second year in Germany and reported that the immunogenicity was adequate, whereas reduced amounts of antigen induced lower antibody concentrations (22). Hendrikx et al. examined IgG responses in children following revaccination at age 4 and found that a booster vaccine with higher pertussis antigen levels induced higher antibody levels than a vaccine containing low antigen levels (23). Okada et al. compared 0.2 and 0.5 ml of DTaP in children aged 11 to 12 years in Japan and observed that GMC and seropositivity rates were similar between the groups (14). Blatter et al. reported that GMCs for anti-PT and anti-FHA were approximately 2-fold higher in the Boostrix group than in the Adacel group 1 month after vaccination, and these differences remained apparent even 1 year after vaccination, although the magnitudes of difference decreased (18). It is difficult to know which dose is appropriate for booster vaccinations. On one hand, a lower dose might be suited to booster vaccinations, as it induces seropositivity levels of antigens with a lower rate of local reactions. On the other hand, a higher dose might be better if it induces not only higher immunogenicity but also longer persistence of this immunogenicity. Studies have demonstrated that antibody concentrations 1 month after vaccination strongly predicted antibody persistence (24) and hence, a higher dose vaccine may contribute to longer persistence of the antibody. However, to our knowledge, there is no study that has compared the long-term persistence of antibodies induced by various doses of antigens.

In the United States, the Advisory Committee on Immunization Practices (ACIP) recommends a Tdap vaccine booster dose for all adolescents aged 11 to 18 years, ideally at 11 to 12 years, and for adults aged 19 to 64 years who have not received a dose since 2005 (25, 26). In 2010, ACIP further recommended a booster dose of Tdap for unvaccinated adults aged 65 years and older who are in close contact with an infant aged <12 months (27). In 2012, ACIP approved the use of Tdap for all adults aged 65 years and older (28). In comparison, no additional DTaP vaccine after a single booster dose in childhood is recommended in Japan. We suspected that the lack of immunization with DTaP during adolescence at 11 to 12 years of age may be the leading cause of the recent resurgence of pertussis among young adults in Japan. To reduce the incidence of infant death as a result of severe pertussis, vaccination among pregnant women may have a greater impact than vaccination among adolescents; however, alteration of vaccination schedules is very difficult. Therefore, adolescents in Japan are currently expected to receive a low dose of DTaP instead of DT (14). However, this may increase the susceptibility to pertussis among adults, including pregnant women, because the immunity induced by DTaP decreases with time. In addition to vaccination in adolescents, repeat booster vaccinations of DTaP may be required to substantially reduce or eliminate the incidence of pertussis. With regard to longer persistence of immunity, a higher dose of booster vaccination may be suitable. Furthermore, the development of a more immunogenic and efficacious pertussis vaccine that requires considerably fewer doses and induces long-term durable protective immunity is required (29).

The present study had several limitations. First, although our relatively small sample size was sufficient to evaluate immunogenicity, it was less than ideal for the detection of adverse events. However, much larger clinical trials investigating Tdap have also not reported serious adverse events (12, 13). Second, study subjects were restricted to young adults only. In the present study, we enrolled students who were exposed to a pertussis outbreak in the previous year and did not exclude individuals who had been exposed to pertussis or had contact with pertussis patients. This may have influenced the vaccination response, although we excluded subjects who developed pertussis in the previous year, after which no outbreak was noted. A healthy group of individuals who were not previously exposed to pertussis would be a better cohort to assess the actual vaccination response in a population experiencing waning immunity from the last vaccination in infancy. However, several outbreaks of pertussis have been reported at college and university campuses in the current decade, and therefore, we consider individuals in this age group as one of the target populations for control of pertussis in Japan. In addition, booster immunization is considered essential at any age in those who have not received it previously. Further studies in other age groups and specific risk groups, such as pregnant women, their newborns, and health care workers, are needed.

In conclusion, 0.2 and 0.5 ml of the DTaP vaccine can induce

antibodies in young adults without severe adverse reactions that affect their daily ordinary activities; thus, both doses can be used for booster immunizations to control pertussis in Japan.

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