pregnant woman acquires rubella virus infection [7] was calculated from two studies conducted in the U.K. [8] and the U.S. [9] (Table 1).

Table 1: Congenital Rubella Syndrome incidence (%) based on the fetal gestational age in pregnant women with rubella virus infection

	U.K.study					U.S. study				
Gestational age (weeks)	≤10	11-12	13-14	15-16	≥17	≤4	5-8	9-12	13-16	≥17
CRS incidence (%)	90	33	11	24	0	70	40	25	40	8

Table 1 Note: Data are from studies conducted in the U.K.[8] and the U.S.[9] .

These three components suggest that the expected number of CRS cases during period t should be $\sum_{i=1}^{n} Prob(\text{CRS of } i \text{ at } t)$, where n is the total number of rubella-infected women, which represents the magnitude of the ongoing rubella outbreak, and $Prob(\text{CRS of } i \text{ at } t)$ is defined as , $q(a(i))\frac{1}{39}p(t-r(i))$ where a(i) is age of patient i, which represents the age distribution of female rubella patients with age 15–49, q(□) is the probability of pregnancy by age i, p(□) is the probability of CRS by gestational age when pregnant and rubella infected and $p_{(\cdot)}=0 \ if \ t-r(i)\geq 40$,and r(i) is the date of implantation of i, where $t-r(i)$ is gestational age.

Hereafter, $\sum_{i=1}^{n} Prob(\text{CRS of } i \text{ at } t)$ is referred to as the CRS potential. Therefore, the CRS potential is defined as the theoretical predicted number of CRS cases based on a rubella outbreak in women. However, the CRS potential might not be equal to the number of reported CRS cases because of under-reporting of rubella and CRS cases or asymptomatic cases. To bridge these gaps, we regressed the number of reported CRS cases based on the CRS potential at birth, $(CRS\ case)_t=\alpha+\beta(CRS\ potential)_t$, by the ordinary least squares method, which is the simplest regression procedure, in which parameters are estimated to minimize the sum of the square of the residual. Data period for estimation was between week 1, 2011 and week 40, 2013.

Finally, to verify the precision of prediction, we performed an ex post evaluation, which is an evaluation of the future based on data available at the time of estimation. Thus, we used data only up to week 40 of 2013 to predict up to week 20of 2014, and compared this with the actual CRS incidence from week 41, 2013 to week 20, 2014.

Results

Following a relatively small outbreak in 2012, a larger outbreak occurred in 2013 (Figure 1). Until week 20, 2014, 3021 rubella cases occurred among women 15–49 years old and 44 CRS cases were reported in accordance with the Infectious Disease Law. The numbers of rubella cases and CRS cases were peaked at week 19, 2013 and at week 2, 2014, respectively. While the CRS potential based on the U.K. study demonstrated a biphasic curve with a low peak around week 12 and a high peak around week 50 of 2013 (Figure 2), the CRS potential based on the U.S. study demonstrated a single peak around week 50 of 2013.

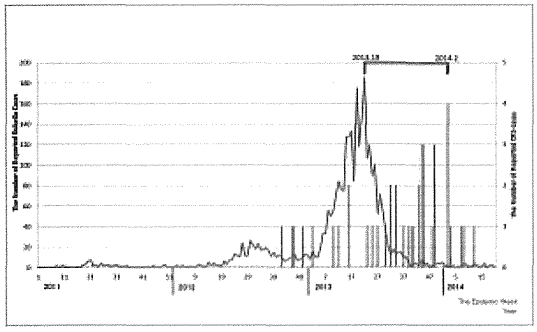

Fig. 1: The number of reported rubella cases (blue line) in women 15-49 years old and congenital rubella syndrome (CRS) cases (red bar) in Japan between week 1, 2011 and week 2, 2014.

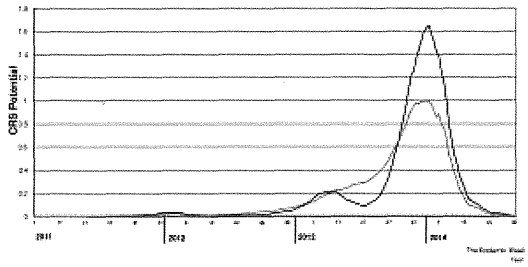

Fig. 2: The congenital rubella syndrome (CRS) potential based on the studies conducted in the U.K. (black line) and the U.S. (green line).

The estimated parameters for the predicted number of CRS cases are shown in Table 2. The estimated b was 0.99 based on the U.K. study and 1.46 based on the U.S. study. The estimated bs indicate that the number of CRS cases is the same as the CRS potential or 1.5 times greater than the CRS potential.Table 2: Estimation of parameters for the predicted number of Congenital Rubella Syndrome cases.

Table 2: Estimation of parameters for the predicted number of Congenital Rubella Syndrome cases

		Estimated coefficient	t-value	p-value
U.K.	α	0.0821	1.80	0.073
	β	0.993	8.77	0.000
U.S.	α	0.0158	0.34	0.735
	β	1.463	9.69	0.000

Table 2 Note: The determinant coefficients, an appropriate index for a fitness of the estimation, of both estimations are 0.330 for the upper panel and 0.376 for the lower panel. Data period for estimation was since week 1,2011 to week 40, 2013.

Figure 3 shows the predicted value, the estimated α + the estimated β (CRS potential), during the period covered by the data used for estimation, which allows prediction of the actual data before week 2, 2014. The predicted cumulative number of CRS cases based on the U.K. and U.S. studies is similar, and both estimations provide a close fit with the actual cumulative number of CRS cases.

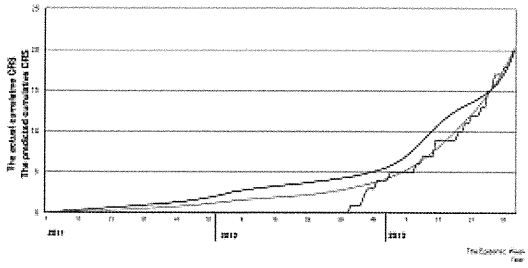

Fig. 3: Comparison between the actual cumulative number of congenital rubella syndrome (CRS) cases (red line) and the predicted cumulative number of CRS cases. The black line and green line are the predicted cumulative numbers of CRS cases based on the studies conducted in the U.K. and the U.S., respectively, using the data up to week 40, 2013.

The cumulative number of CRS cases in the future at the time of prediction, i.e., the period after week 2, 2014, is shown in Figure 3. The cumulative number of CRS cases in 2014 is predicted to be 19.1–29.3.

The results of the ex post evaluation are shown in Figure 4. The curve of the predicted cumulative number of CRS cases based on the U.S. data is**close to**the curve of the actual cumulative number of CRS cases between week 40, 2012 and week 40, 2013, while the curve of the predicted cumulative number of CRS cases based on the U.K. data is slightly higher than the curve of the actual cumulative number of CRS cases during the same period. After week 47, 2013, the curve of the predicted cumulative number of CRS cases based on the U.S. data is close to the actual cumulative number of CRS cases **until around week 5, 2014.** In contrast, the curve of the predicted cumulative number of CRS cases based on the U.K. data is much higher than the curve of the actual cumulative number of CRS cases.

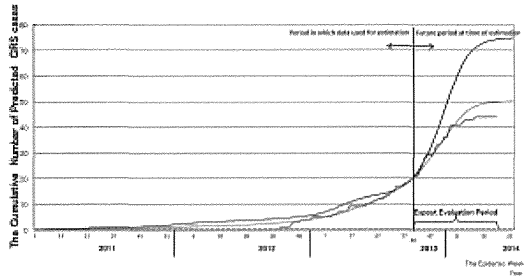

Fig. 4: Ex post evaluation of the predicted cumulative numbers of congenital rubella syndrome (CRS) cases. The red line indicates the actual cumulative number of CRS cases up to week 2, 2014. The black line and green line are the predicted cumulative numbers of CRS cases based on the studies conducted in the U.K. and the U.S., respectively, using the data up to week 40, 2013.

Discussion

In this study, we predicted the number of CRS cases with a formulation based on the parameters from two studies from the U.K. and the U.S. in 2013 and up to week 25, 2014. We found an increase of CRS cases following the rubella epidemic with a lag of 5-7 months, which was almost in agreement with a previous experience in Greece [4] . A close correlation was found between the predicted cumulative number of CRS cases in 2013 and the actual cumulative number of reported CRS cases, validating the present method of CRS prediction (Figure 3). The ex post evaluation generated two different patterns of the predicted number of CRS cases: a higher prediction based on parameters from the U.K. study and a lower prediction based on the U.S. study (Figure 4). The lower prediction based on the data from the U.S. agreed closely with the actual cumulative number of CRS cases. A dissociation between the predicted number of CRS cases based on two studies from the U.K. and the U.S. may be attributable to a difference in the probability of CRS, which directly affects the CRS potential, during the period up to 10 weeks of gestational age. While a higher percentage (90%) of CRS was found during the period up to 10 weeks of gestational age in the U.K. study, relatively lower percentages (40% or 70%, respectively) of CRS, compared with that in the UK study, during the period up to 10 weeks or between 5 and 8 weeks of gestational age were observed in the U.S. study.However, the reasons for the difference of the CRS incidence between the US and UK epidemics remain uncertain from the two literatures[8,9] . Actually, the estimation of β based on the past data retrospectively and thus it may not reflect the current or future situation. However, as shown in Figure 3 and ex post evaluation, it was proved to have a quite preciseness for the prediction of CRS and it would be valuable for public health workers.

CRS is clinically confirmed if an infant has: 1) at least two of cataract, congenital glaucoma, congenital heart disease, hearing impairment, or pigmentary retinopathy; or 2) one of these complications, and one of purpura, splenomegaly, microcephaly, meningoencephalitis, radiolucent bone disease, or jaundice developed within 24 hours after birth [3] . A cataract, which is found in approximately one third of all CRS babies, is occasionally not observed until late infancy [10] . Sensorineural deafness is also the most common manifestation of CRS, and deafness is frequently overlooked in infancy. Therefore, the difficulties in the clinical diagnosis of CRS often cause a delayed notification of CRS cases, and temporarily decrease the number of reported CRS cases compared with that predicted by our method.

In summary, we predicted the cumulative number of CRS cases in 2014 by a formula based on the parameters of two studies from the U.K. and the U.S. Our method for prediction of the number of CRS cases may be useful

for the enhanced detection of this syndrome that is often under-reported.

Competing Interest

The authors have declared that no competing interests exist.

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YO is a senior scientist of the Infectious Disease Surveillance Center, National Institute of Infectious Diseases, Japan, and works in biostatistics, cost-effectiveness, and mathematical modelling.

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Comparison of the immunogenicity and safety of polysaccharide and protein-conjugated pneumococcal vaccines among the elderly aged 80 years or older in Japan: An open-labeled randomized study

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ABSTRACT

An open-labeled randomized study was conducted to compare the immunogenicity and safety of polysaccharide (PPV23) or protein-conjugated pneumococcal vaccine (PCV7) among the elderly aged 80 years or older. A total of 105 nursing home residents were enrolled in this study. We analyzed the geometric mean concentration (GMC) of serotype-specific immunoglobulin G (IgG) and the geometric mean titer (GMT) of the opsonization index (OI) for serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. The GMCs of serotype-specific IgG and the GMTs of the OI significantly increased one month after vaccination in both groups for all seven serotypes evaluated. In the PCV7 group, study subjects with serotypes 4, 9V, 18C, and 23F exhibited statistically significant elevations in both serotype-specific IgGs and OIs compared to those of the PPV23 group. Both vaccines were tolerated without any severe adverse events, and no differences in systemic adverse events were observed between the two groups, although adverse reactions such as redness and localized swelling were more common in the PCV7 group. Our data demonstrated that the GMCs of serotype-specific IgG and the GMTs of the OI were higher in the PCV7 group compared to those in the PPV23 group. Our study also confirmed the safety of both the PCV7 and PPV23 vaccines in elderly people aged 80 years or older.

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1. Introduction

Streptococcus pneumoniae infection is a major cause of mortality and morbidity worldwide among the elderly. The 23-valent pneumococcal polysaccharide vaccine (PPV23) is widely recommended for administration to those who are at a high risk of *S. pneumoniae* infection, such as elderly people and splenectomy patients [1]. However, owing to the purified free polysaccharides that comprise its surface capsule, PPV23 does not elicit T cell-dependent immune responses and is a poor inducer of immunologic memory. Furthermore, vaccine-induced antibody titers may achieve insufficient levels and decrease annually, particularly 5 years after vaccination [2].

The conjugation of the capsular polysaccharide to a diphtheria protein stimulates not only B-cell immune response but also T cell-dependent immune responses and enhanced memory response at the time of boosting [3]. Therefore, pneumococcal conjugate vaccines produce superior immune responses, particularly in infants. For this reason, the heptavalent pneumococcal conjugate vaccine (PCV7) was licensed in 2000 in the United States and in 2009 in Japan. PCV7 also produces better immune responses than PPV23 in groups at higher risk of developing invasive pneumococcal diseases

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and pneumococcal pneumonia, such as individuals with HIV [4] or chronic obstructive pulmonary disease [5].

In healthy elderly people 50–80 years old, Goldblatt et al. [6] reported that PCV7 produced superior immunogenicity compared with PPV23. In recent years, the increasing number of elderly people over 80 years old hospitalized for pneumococcal pneumonia has been reported [7]. While pneumococcal vaccination is strongly recommended for this population, no data are currently available for comparison of the immunogenicity and safety between PCV7 and PPV23 for this age group. Therefore, we performed this prospective study to clarify these unknown aspects.

2. Materials and methods

2.1. Study subjects

The present study was a randomized, open-label study designed to compare the immunogenicity and safety of PCV7 (Prevenar; Pfizer) with those of PPV23 (Pneumovax; MSD). Data were collected between April 2011 and December 2012 from participants who were 80 years or older and had never received pneumococcal vaccinations. None of the participants had any documented history of pneumococcal infection. They were selected from five different nursing homes around Tokyo and were randomly assigned to either the PPV23 group or the PCV7 group using the sealed envelope system with a 1:1 allocation ratio. A total of 105 participants were enrolled in this study, and all participants provided written informed consent.

In addition, subjects were excluded if they had a history of any streptococcal vaccination, a history of anaphylactic reaction to diphtheria toxin, or symptoms of fever on the day of vaccination.

We set the sample size on the basis of a study by Goldblatt et al. [6] on the comparison of immunogenicity between PCV7 and PPV23 among adults aged 50–80 years. They assigned 33–60 subjects to a subgroup of one arm and showed higher geometric mean concentrations (GMCs) of serotype-specific IgG response in several serotypes.

This study was reviewed and approved by the Research Ethics Committee of Keio University School of Medicine (2010-231-2) and by the Research Ethics Committee of Kitasato University Kitasato Institute Hospital (1108-02). This trial was registered with the UMIN Clinical Trials Registry (UMIN000006132).

2.2. Vaccines

The PCV7 used in this study is currently licensed only for pediatric use in Japan. PCV7 contains polysaccharides of pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F, which are conjugated to the protein carrier CRM197, a nontoxic variant of the diphtheria toxin. Each serotype-specific polysaccharide is conjugated separately prior to formulation as a multivalent vaccine. The vaccine contains aluminum phosphate as an adjuvant.

PPV23 contains a mixture of purified capsular polysaccharides from 23 different serotypes of *S. pneumoniae*: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F. This vaccine is adjuvant-free.

Each participant received 0.5 mL of either PPV23 or PCV7 via subcutaneous injection. PPV23 and PCV7 were dispensed and administered by members who were not blinded and not involved in subsequent data analysis.

2.3. Samples

Blood samples (10 mL) were drawn from all the subjects on the day of vaccination and approximately one month after vaccination. Sera were separated by centrifugation (3500 rpm, 15 min, 4 °C) and stored at −80 °C.

2.4. Enzyme-linked immunosorbent assay (ELISA)

Anti-pneumococcal immunoglobulin G (IgG) antibodies were measured by World Health Organization (WHO)-approved ELISA, using standard reference serum (89-SF or 007sp) and C-polysaccharide and 22F polysaccharide absorption, as previously reported [8,9]. The levels of serotype-specific IgGs for seven serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) were determined in both vaccination groups according to the WHO protocol (a detailed version of the protocol is available at http://www.vaccine.uab.edu/ELISAProtocol(89SF).pdf). These serotypes are covered by PCV7.

2.5. Multiplexed opsonophagocytic killing assay

A multiplexed opsonophagocytic killing assay for seven serotypes, 4, 6B, 9V, 14, 18C, 19F, and 23F, based on antibiotic-resistant strain target bacteria, was performed at the Research Institute for Microbial Diseases, Osaka University, as previously described [10]. The quality control serum used in each assay was prepared from the pooled sera of adults vaccinated with PPV23 or PCV7. The opsonization index (OI) was defined as the serum dilution capable of killing 50% of the bacteria, which was determined by using opsotiter3 software according to the WHO protocol (a detailed version of this protocol is available at www.vaccine.uab.edu/UAB-MOPA.pdf) [11]. Laboratory analysis, ELISA, and a multiplexed opsonophagocytic killing assay were performed by members who were blinded to vaccine allocation.

2.6. Adverse reactions

All patients were observed daily by medical staff to monitor body temperature and any local or systemic reactions, starting from the day of vaccination to day 7. Injections were graded based on the occurrence of several possible adverse events as follows: grade I (the reaction was present but easily tolerated), grade II (the reaction interfered with normal activity), and grade III (the reaction was severe or incapacitating).

2.7. Statistical analysis

Average antibody concentrations and the increases from baseline were expressed as geometric means. Differences in the GMCs of serotype-specific IgG and the geometric mean titers (GMTs) of the OI were assessed by the Wilcoxon matched-pairs signed-ranks test. For multiple comparisons, we calculated Bonferroni-adjusted *P* values. The frequencies of adverse reactions were compared between vaccinations by the Fisher exact test. Differences with $P < 0.05$ were considered to be statistically significant. Data analysis was performed by members who were blinded to vaccine allocation.

3. Results

3.1. Participant characteristics

Overall, 623 eligible participants were reviewed in the 5 nursing homes (Fig. 1). One hundred and five participants were enrolled in this study after they provided written informed consent. Five subjects were subsequently dropped from the study prior to vaccination (2 subjects were hospitalized, 2 subjects left the nursing home, and 1 subject died). Consequently, 100 subjects were vaccinated (Table 1); of these, 49 received PPV23 and 51 received PCV7. The mean ages at enrollment were 88.3 years for the PPV23 group and 87.7 years for the PCV7 group, with 45 subjects in their

Table 1
Participants characteristics.

Characteristics	PPV23[a] (n = 49)	PCV7[b] (n = 51)	P value
Age, years	88.3 ± 1.4	87.7 ± 1.5	0.29
Male, %	12 (24.5)	11 (21.6)	
Female, %	37 (75.5)	40 (78.4)	
Height, cm	145.1 ± 3.9	146.5 ± 2.2	0.24
Weight, kg	45.9 ± 2.4	46.0 ± 2.1	0.47
Hypertension, %	34 (69.4)	32 (62.7)	
Diabetes mellitus, %	15 (30.6)	18 (35.2)	
Old cerebral infarction, %	17 (34.7)	14 (27.5)	
Dementia, %	15 (30.6)	13 (25.5)	
Dyslipidemia, %	14 (28.6)	12 (23.5)	
Neck of femur fracture, %	12 (24.5)	13 (25.5)	
Congestive heart failure, %	12 (24.5)	10 (19.6)	
Vertebral compression fracture, %	11 (22.4)	9 (17.6)	
Cataract, %	6 (12.2)	5 (9.8)	
Chronic obstructive pulmonary disease, %	6 (12.2)	3 (5.9)	
Old myocardial infarction, %	5 (10.2)	5 (9.8)	
Malignancy, %	4 (8.1)	5 (9.8)	
Benign prostatic hyperplasia, %	5 (10.2)	3 (5.9)	
White blood cells, counts/μl	5796 ± 398	6101 ± 431	0.15
Hemoglobin, g/dl	11.8 ± 0.4	12.2 ± 0.4	0.09
Platelets, $\times 10^4$ counts/μl	23.6 ± 2.2	22.8 ± 2.0	0.28
Albumin, g/dl	3.7 ± 0.1	3.7 ± 0.1	0.35
AST, IU/l	20.7 ± 2.0	21.1 ± 2.9	0.40
ALT, IU/l	12.9 ± 2.0	13.5 ± 2.5	0.35
BUN, mg/dl	17.6 ± 1.0	16.5 ± 1.3	0.10
Creatinine, mg/dl	0.68 ± 0.5	0.70 ± 0.5	0.25

Data are presented as mean ± SD (standard deviation) unless otherwise indicated.
[a] 23-valent pneumococcal polysaccharide vaccine.
[b] 7-valent pneumococcal conjugate vaccine.

90s and 3 subjects who were 101 years old. The majority (77%) of the subjects were female. There were no significant differences in major co-morbidities between the PPV23 group and the PCV7 group. No other significant differences in laboratory data were observed between the two groups. All the participants from both groups received routine immunization against seasonal influenza.

3.2. Immunogenicity: levels of serotype-specific IgG

Data for the GMCs of serotype-specific IgG responses before and one month after vaccination with PPV23 or PCV7 are summarized in Table 2 and presented graphically in Fig. 2. The original data on serotype-specific IgG are also shown in Supplementary Table 1. No significant differences of baseline serotype-specific IgG GMCs were observed between the two groups for all serotypes measured. In both groups, significant increases in IgG GMCs were observed from baseline to one month following the initial dose for all seven serotypes evaluated. The GMCs of serotype-specific IgGs for serotypes 4, 9V, 18C, and 23F of the study subjects were significantly more elevated in the PCV7 group than in the PPV23 group.

3.3. Immunogenicity: OI

Data for the GMTs of serotype-specific OIs before and one month after vaccination with PPV23 or PCV7 are summarized in Table 3 and presented graphically in Fig. 3. The original data on serotype-specific OIs are also shown in Supplementary Table 2. No significant differences in the baseline GMTs of serotype-specific OIs were observed between the two groups for all serotypes measured. In both groups, significant increases in the GMTs of OIs were observed from baseline to one month following the initial dose for all seven serotypes evaluated. The GMTs of serotype-specific OIs for serotypes 4, 9V, 18C, and 23F of the study subjects were significantly elevated in the PCV7 group compared to the PPV23 group.

3.4. Safety

Both vaccines were tolerated without any severe adverse events. No differences were observed in systemic side effects between the two groups; however, local side effects such as redness and localized swelling were more commonly observed in the PCV7 group (Table 4). No participants required unscheduled medical examinations within the first 7 days after vaccination.

4. Discussion

The current study is the first to demonstrate pneumococcal vaccine responses in pneumococcus vaccine-naïve elderly people (at or over 80 years of age) by evaluating serotype-specific IgG antibodies and serotype-specific OIs between PPV23 and PCV7. Our major findings are that both PPV23 and PCV7 elicited increases in IgG and OI, and that PCV7 is more potent than PPV23 in terms of its immunogenicity against four out of seven serotypes included in PCV7. We also demonstrated the safety of these preparations in these elderly individuals, with no serious adverse effects observed in either group.

We believe that there are several important strengths of this study. One of them is that not only serotype-specific IgG levels but also serotype-specific OIs were evaluated. Due to technical difficulties with OI assays, OI measurements have been reported in only a limited number of clinical studies to date. However, we were able to evaluate functional antibodies, which are superior surrogate markers for protection against pneumococcal pneumonia and bacteremia, by utilizing the latest generation of ELISA methodology [12].

Another important strength of this study is the age distribution of the participants, considering the current inevitable tendency toward increasing longevity in humans. Since the host response induced by vaccinations varies depending on the age of the recipient, the development of safe and effective vaccinations for the elderly is clinically important.

In our study, antibodies against serotypes 4, 9V, 18C, and 23F were significantly elevated in the study subjects. These data were consistent with a previous study indicating that serotype-specific IgG levels of 4, 6B, 9V, 14, 18C, and 23F, and serotype-specific OIs of 4, 9V, 14, 18C, and 23F were significantly elevated in the PCV7 group consisting of elderly people more than 70 years old [2]. In addition, in accordance with our data, they reported that serotype 6B and 19F did not show superior immunogenicity compared with other serotypes in elderly people.

In several studies, 1.0-mL doses of PCV7 were administered [5,13]. However, we used half this dosage in our clinical study to minimize potential adverse effects. In fact, both PPV23 and PCV7 were tolerated by the participants and were associated with few local reactions or systemic adverse effects. No severe adverse effects were observed in either group. A higher frequency of local reactions was observed in the PCV7 group compared with the PPV23 group, although we were unable to determine if this increase was caused by the conjugation specifically. According to a dose-range study of pneumococcal conjugate vaccine reported by Lode et al. [14], both serotype-specific IgG and OI displayed increases in dose-dependent manners, although local reactions for the double dose were not statistically higher than for the single dose. Based on this notion, in our study, 1.0-mL injections rather than

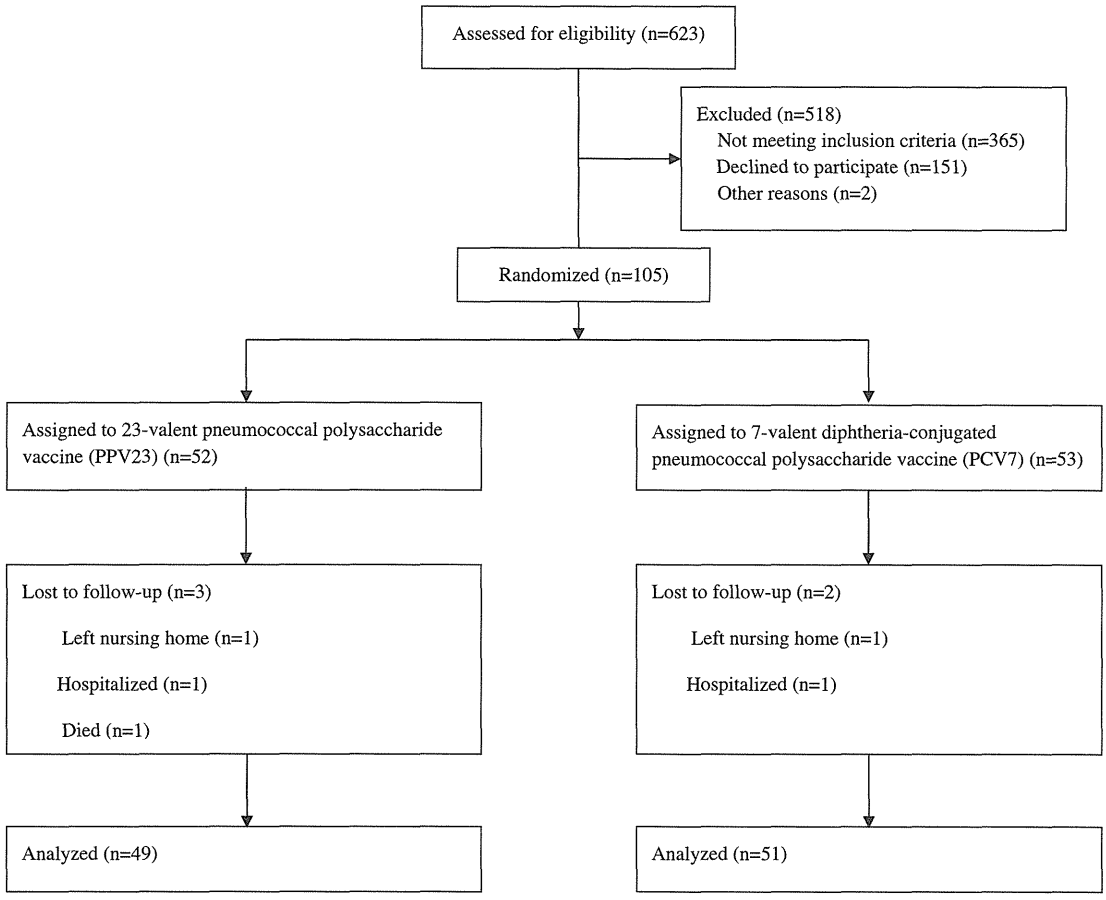

Fig. 1. Flow diagram of trial.

0.5-mL injections of PCV7 could have safely obtained more potent immunogenicity.

The mean number of co-morbidities of study participants staying at nursing homes was 3.34 in our study. According to the nationwide epidemiological study in Scotland by Barnett K et al., the mean number of co-morbidities is 2.60 in elderly people aged 65–84 years and 3.62 in elderly people aged 85 and over [15]. The cross sectional study of aged Medicare beneficiaries in the United States shows that the mean number of co-morbidities is 2.71 in elderly people aged 80 and over [16]. Considering these previous data, our study population of nursing homes could be regarded as not an unusual population of the elderly people in the developed countries.

This study has several limitations. Firstly, a vaccine type and an injection route have to be considered. In this study, we could not use PCV13 because of the lack of the license in Japan at the time of the current study, while PCV13 was launched in Japan in 2014. Therefore our study is out-of-date data at the present. Although PCV is usually administered intramuscularly, not subcutaneously, an intramuscular injection of PCV7 was not allowed in Japan at the time of our clinical study. In order to minimize injection dependent bias, we administered PCV7 subcutaneously by following that the most common route of PPV23 is the subcutaneous route in Japan. Even though, we should have administered both PPV23 and PCV7 intramuscularly.

Table 2
The geometric mean concentrations of serotype-specific IgG antibody before and one month after vaccination pneumococcal vaccines.

Serotype	Pre IgG μg/ml (95% CI)		Post IgG μg/ml (95% CI)		Bonferroni-adjusted *P* value
	PPV23[a] (*n* = 49)	PCV7[b] (*n* = 51)	PPV23 (*n* = 49)	PCV7 (*n* = 51)	
4*	0.44 (0.35–0.55)	0.52 (0.42–0.66)	1.02 (0.77–1.34)	3.38 (2.32–4.92)*	>0.001
6B	1.22 (1.00–1.64)	1.11 (0.84–1.39)	3.51 (2.66–5.30)	3.32 (2.08–4.84)	6.205
9V*	1.03 (0.81–1.38)	0.92 (0.70–1.18)	4.01 (3.12–5.66)	8.75 (5.80–12.14)*	0.003
14	1.88 (1.44–2.85)	2.26 (1.61–3.22)	7.66 (5.00–14.02)	11.41 (7.57–18.26)	3.723
18C*	1.12 (0.89–1.56)	1.08 (0.80–1.39)	4.93 (3.53–6.76)	10.02 (6.98–14.39)*	0.043
19F	1.69 (1.38–2.15)	2.24 (1.72–2.82)	5.26 (3.65–7.30)	6.10 (4.08–8.45)	2.467
23F*	1.28 (0.95–1.81)	1.31 (0.95–1.80)	5.39 (3.51–8.98)	14.68 (9.75–22.04)*	0.014

[a] 23-valent pneumococcal polysaccharide vaccine.
[b] 7-valent pneumococcal conjugate vaccine.
* A significant difference in absolute postvaccination IgG levels between vaccine groups.
Within each study group, postvaccination antibody levels were higher than baseline ($P < 0.01$) for all serotypes.

Table 3
The geometric mean titers of serotype-specific opsonization index before and one month after vaccination pneumococcal vaccines.

Serotype	Pre OI[a] (95% CI)		Post OI (95% CI)		Bonferroni-adjusted *P* value
	PPV23[b] ($n=49$)	PCV7[c] ($n=51$)	PPV23 ($n=49$)	PCV7 ($n=51$)	
4*	3.55 (2.55–5.22)	5.77 (3.53–9.44)	45.84 (25.55–104.83)	710.65* (307.45–1642.62)*	0.005
6B	17.97 (10.58–38.69)	23.34 (12.10–40.88)	271.51 (123.06–586.19)	700.27 (327.87–1188.63)	2.227
9V*	24.44 (13.77–49.77)	19.34 (9.97–34.30)	234.47 (138.37–478.25)	958.78* (559.49–1680.79)*	0.012
14	44.83 (24.67–101.30)	90.57 (38.43–183.84)	588.67 (262.75–1380.93)	1925.23 (1144.17–3430.09)	2.259
18C*	47.67 (25.48–89.65)	39.13 (19.53–69.75)	708.20 (329.96–1295.19)	2730.37* (1805.42–4118.33)*	0.016
19F	18.26 (10.23–32.94)	25.94 (13.43–45.32)	352.42 (163.69–628.10)	414.32 (196.85–707.47)	3.572
23F*	19.00 (10.50–35.76)	14.51 (7.30–26.69)	197.51 (80.78–466.58)	2076.51* (1129.01–3937.53)*	>0.001

[a] Opsonization index.
[b] 23-valent pneumococcal polysaccharide vaccine.
[c] 7-valent pneumococcal conjugate vaccine.
* Bolded items represent a significant difference in absolute postvaccination OI levels between vaccine groups.
Within each study group, postvaccination OI were higher than baseline ($P<0.01$) for all serotypes.

Another limitation is that it was only one month after vaccination that the antibody levels were examined, thereby limiting our knowledge regarding long-term effects. Our recent study suggested sustained levels of serotype-specific IgG and OI after primary and secondary vaccination with PPV23 among elderly individuals with chronic lung diseases [17]. We therefore intend to compare the serotype-specific IgG and OI after primary vaccination between the study subjects immunized with PPV23 and PCV7 in this study.

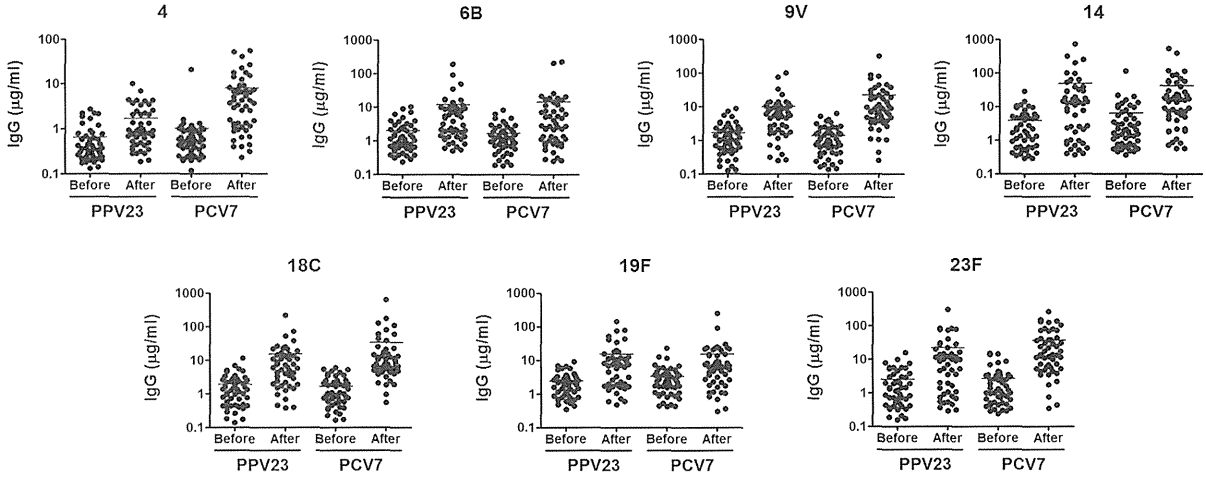

Fig. 2. The serotype-specific baseline and 1-month absolute IgG antibody levels are shown for each patient. The heptavalent diphtheria-conjugated pneumococcal polysaccharide vaccine (PCV7) resulted in statistically significantly higher antibody levels at one month to baseline for serotypes 4, 9V, 18C and 23F.

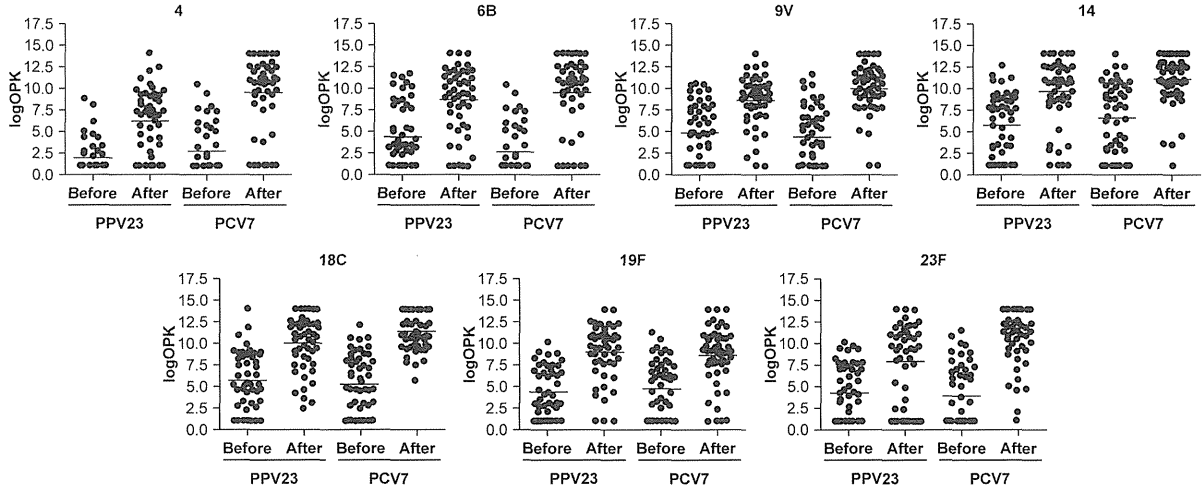

Fig. 3. The serotype-specific baseline and one-month geometric mean opsonophagocytic killing index are shown for each patient. The heptavalent diphtheria-conjugated pneumococcal polysaccharide vaccine (PCV7) resulted in statistically significantly higher geometric mean opsonophagocytic killing index at one month to baseline for serotypes 4, 9V, 18C and 23F.

Table 4
Comparison of adverse reactions among elderly individuals after vaccination with pneumococcal vaccines.

Characteristics	PPV23[a] (n=49)	PCV7[b] (n=51)	P value
Fatigue			
Grade I	3	3	0.96
Grade II	0	0	-
Muscle aches			
Grade I	0	0	-
Grade II	0	0	-
Headache			
Grade I	0	0	-
Grade II	0	0	-
Itching of vaccinated arm			
Grade I	2	4	0.43
Grade II	0	0	-
Pain of vaccinated arm			
Grade I	0	0	-
Grade II	0	0	-
Fever			
Grade I	4	3	0.65
Grade II	0	0	-
Redness			
Grade I (<8 cm)	9	16	0.13
Grade II (>8 cm and <15 cm)	3	5	0.50
Grade III (>15 cm)	0	0	-
Localized swelling			
Grade I (<8 cm)	11	19	0.11
Grade II (>8 cm and <15 cm)	0	0	-

[a] 23-valent pneumococcal polysaccharide vaccine.
[b] 7-valent pneumococcal conjugate vaccine.

There are several unsolved issues for pneumococcal vaccination. The titer of correlate of protection for adults who received pneumococcal vaccines has not yet been established, while a titer of 0.35 μg/mL has been defined as a correlate of protection against invasive diseases among infants who received the pneumococcal conjugate vaccine. In this respect, as for adults, the advantage of the higher immunogenicity in the PCV7 group is not clear in protection against pneumococcal diseases. Moreover, the difference of the serotypes covering range by each pneumococcal vaccine has to be taken into consideration. Based on the newest domestic reports on the serotype distribution of community-acquired pneumonia (CAP) [18] and invasive pneumococcal disease (IPD) [19], the ratio of serotypes of CAP covered by PPV23, PCV7 and PCV13 are 82.5%, 61.4% and 83.3% while the ratio of serotypes of IPD covered by PPV23, PCV7 and PCV13 are 85.4%, 39.8% and 61.9%, respectively. Taken together, to make best of our current study, the nationwide surveillance of *S. pneumoniae* infections is essential in Japan. Beyond the scope of this current study, the most important aspect is to establish the vaccine policy which produce clinical efficacy for preventing *S. pneumoniae* infections.

In conclusion, we demonstrated higher increases in the GMCs of serotype-specific IgG levels and the GMTs of OIs in the PCV7 group compared to the PPV23 group, and confirmed the safety of vaccinations with PCV7 and PPV23 for subjects aged 80 years and older.

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Conflict of interest: Dr. Hasegawa has received grants from MSD and Pfizer.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2014.11.023.

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Original article

IgG levels against 13-valent pneumococcal conjugate vaccine serotypes in non pneumococcal conjugate vaccine immunized healthy Japanese and intravenous immunoglobulin preparations

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ABSTRACT

No studies showed specific antibody levels against all serotypes covered by 13-valent pneumococcal conjugate vaccine (PCV13) among polyclonal intravenous immunoglobulin (IVIG) products. Our study aimed to assess whether we could expect the efficacy of IVIG therapy for invasive pneumococcal disease (IPD) and to clarify the age group which should be recommended for IVIG therapy in case of IPD. Serotype-specific immunoglobulin G (IgG) levels against PCV13 serotypes were measured in four IVIGs which were produced from Japanese donors who were not immunized with any pneumococcal conjugate vaccines (PCVs), and in the serum of 160 non-PCV immunized Japanese subjects, by enzyme-linked immunosorbent assay. The functional opsonic activities of the IVIGs against serotypes 6B and 19A were assessed by a multiplexed opsonophagocytic killing assay. Japanese infants aged <2 years had a geometric mean IgG concentration of <0.35 μg/ml against several serotypes. Serotype-specific IgG concentrations varied among IVIGs. In general, IgG antibodies against serotypes 6A, 14 and 19A were higher in each IVIG. Although opsonization indices also varied among preparations, each IVIG had the ability to opsonize both serotypes 6B and 19A. This study suggests that routine immunization with PCV is important for prevention of IPD, especially for children <2 years old and IVIGs might be effective for IPD patients.

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1. Introduction

Streptococcus pneumoniae, a leading cause of pneumonia, bacteremia, and meningitis, causes substantial morbidity and mortality. Worldwide, *S. pneumoniae* causes an estimated 1.6 million deaths annually, of which about 830,000 occurred among children <5 years of age in the year 2000 [1]. Invasive pneumococcal disease (IPD) sometimes causes fatality and sequelae, especially for people with underlying disease [2].

El-Nawawy et al. reported a randomized controlled study of polyclonal intravenous immunoglobulin (IVIG) in pediatric sepsis syndrome, where patients showed a significant reduction in mortality [3]. IVIG administration might be an effective therapy against IPD. However, little has been reported about IVIG treatment for IPD, except reports about prevention of infections in common variable immune deficiency [4] and patients without a spleen [5].

Food and Drug Administration (FDA) regulations, which also apply to IVIG, require that all immunoglobulin product lots possess a minimum level of antibodies to measles, diphtheria, and poliomyelitis (which are provided in FDA regulation 21 CFR 640.104), but not serotype-specific antibodies to *S. pneumoniae*. In 2000, the World Health Organization (WHO) described guidelines for the pneumococcal enzyme-linked immunosorbent assay (ELISA) as an international standard protocol to quantify the concentrations of antibody levels against pneumococcal serotype-specific polysaccharides (http://www.vaccine.uab.edu). To measure pneumococcal serotype-specific antibody levels against all 13-valent

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pneumococcal conjugate vaccine (PCV13) serotypes by this ELISA would be time-consuming and expensive. A few studies showed specific antibody levels against several selected pneumococcal serotypes among IVIG products [6], and there is no report about serotype-specific antibody levels against all serotypes covered by PCV13, serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F.

IPDs caused by serotypes (especially 19A) not covered by the 7-valent pneumococcal conjugate vaccine (PCV7) increased after the introduction of PCV7 [7]. In Japan, as PCV7 was introduced in 2010, IPDs by non-PCV7 serotypes would be forecast to increase in the future. Adjuvant therapy with antibiotics might be needed for patients with IPDs caused by non-vaccine serotypes. Therefore, it is important to investigate specific antibody concentrations against non-PCV7 pneumococcal serotypes in IVIG products.

In this study, we measured serotype-specific immunoglobulin G (IgG) antibody levels against all pneumococcal serotypes covered by PCV13 (PCV7 serotypes + 1, 3, 5, 6A, 7F and 19A) in commercially available IVIG preparations, to assess whether we could expect the efficacy of IVIG therapy for patients with IPD, and in the general Japanese population, to clarify which age group should be recommended IVIG therapy in case of IPD, using WHO ELISA protocol. In addition, we assessed functional opsonization of serotype 6B and 19A by IVIG preparations using a multiplexed opsonophagocytic killing assay (MOPA), because serotype 6B frequently causes IPD in Japan [2] and IPD caused by serotype 19A is an increasing problem at present.

2. Materials and methods

2.1. Serum samples

The serum samples were collected between 2006 and 2012 from healthy Japanese children and young adults who were not immunized pneumococcal conjugate vaccines (PCVs). Of these samples, 146 samples were collected before the introduction of PCV7 and 14 samples were collected after the introduction of PCV7. The number of samples analyzed per group aged <2 years old, 2–4 years old, 5–9 years old and young adults (18–23 years old) were 38, 21, 51and 50 with respectively. Vaccination with the 23-valent pneumococcal polysaccharide vaccine (PPV23) is only recommended in Japan for risk groups, such as children over 2 years old with high risk underlying diseases for IPD and elderly over 65 years old. PPV23 is not used as a routine vaccination. Although no information about vaccination with PPV23 was available for participants in this study, usage can considered to be rare. Serum samples were stored at <−20 °C until analysis. The study was conducted to comply with the ethical standards of Chiba University on human experimentation, and with the Helsinki Declaration. The informed consent was obtained from subjects or their guardians.

2.2. IVIG preparations

Five lots each of four commercial IVIG preparations (identified here as A to D), were analyzed in this study. Each IVIG product was characterized by different treatments during production, as follows: product A was treated with polyethylene glycol; product B was a freeze-dried preparation treated with polyethylene glycol; product C was an acidic preparation subjected to incubation at pH 4; and product D was a freeze-dried, sulfonated preparation. The IVIG preparations were produced from the plasma of voluntary Japanese blood donors between 2008 and 2011. In Japan, PCV7 was introduced for children under 10 years old in 2010. Blood donors must be at least 16 years old in Japan. Therefore all preparations were produced from the plasma of donors who were not immunized with PCVs. All IVIGs were provided as 50 mg/ml solutions. Reconstituted IVIGs were diluted 1:200 with PBS for the ELISA. When the results were assessed, specific pneumococcal IgG levels in IVIG preparations were re-calculated the experimental data by two hundred times.

2.3. Pneumococcal serotype-specific IgG assay

Pneumococcal serotype-specific IgG was measured with the WHO approved ELISA using the standard reference serum (007sp), and C-polysaccharide and 22F polysaccharide absorptions (http://www.vaccine.uab.edu) as previous reported [8]. Optical density data were converted to antibody concentrations with a computer program which used a four parameter logistic-log method to perform a curve-fitting procedure. Details of the procedure are provided at http://www.vaccine.uab.edu.

We assessed the concentrations of antibodies against all serotypes covered by PCV13, serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F. The WHO suggested a serotype-specific IgG concentration of 0.35 μg/ml as a putative measure of protection at a population level against IPD in infants after immunization with PCV [9]. This reference antibody concentration of ≥0.35 μg/ml has been determined through a pooled analysis of data from the PCV efficacy trials with invasive disease end-points that have been completed to date. This threshold does not necessarily predict protection by naturally acquired immunity in an individual subject. Additionally, the relevance of this threshold to passively acquired immunity by using IVIG has not been established.

We used serotype-specific IgG antibody >0.35 μg/ml as the temporary threshold in this study.

2.4. Multiplexed opsonophagocytic killing assay (MOPA)

In order to measure the functional activity of the anti-pneuomococcal antibodies in the IVIG products, a multiplexed opsonophagocytic killing assay (MOPA) was performed. Opsonization indices (OIs) were measured for serotypes 6B and 19A based on the phagocytosis of antibiotic-resistant target bacteria, as previously described [10]. For total IVIG, the total IgG concentration at the first dilution of the IVIG (1:4) was 12.5 mg/ml. Pneumococcal strains, of serotypes 6B and 19A, resistant to spectinomycin or trimethoprim, respectively, were obtained from BEI Resources (www.beiresources.org). Differentiated HL-60cells (ATCC, Manassas, VA) were allowed to phagocytose *S. pneumoniae* in the presence of IVIG antibodies and baby rabbit complement (Pel-freez Biologicals, Rogers, AR). 10 μl aliquots of the reaction mixture were plated onto two different Todd-Hewitt-0.5% Yeast extract agar plates containing appropriate antibiotics. The bacterial colonies were counted after overnight incubation at 37 °C in an atmosphere containing 5% CO_2. The OI was defined as the greatest dilution of IVIG that killed 50% of bacteria. The OIs were determined using opsotiter3 software according to the WHO protocol (www.vaccine.uab.edu/UAB-MOPA).

2.5. Statistical analysis

Geometric mean IgG concentrations (GMCs) with 95% confidence intervals (CI) were calculated for each age group. The average for the five IVIG lots was shown using bar graphs with error bars representing one standard deviation. All statistical analyses were performed using GraphPad Prism v5 software (GraphPad Software, Inc.). Calculations of *p*-values were performed with one-way ANOVA, followed by Tukey's multiple comparison test, to compare the individual serotype-specific IgG levels for all 13 serotypes in each age group, or each IVIG preparation. Calculations of *p*-values were performed with two-way ANOVA followed by the

Bonferroni post-hoc test, to compare the serotype-specific IgG levels against individual serotypes among IVIG preparations.

3. Results

3.1. Seroprevalence of pneumococcal serotype-specific IgG antibodies in the Japanese population

Fig. 1 illustrates GMCs of the IgG levels for each of the 13 serotypes stratified by age group. Overall, infants aged <2 years had low GMCs. Infants aged <2 years had GMCs <0.35 μg/ml against serotypes 1, 4, 7F, 9V, 18C and 23F. GMCs against all serotypes increased with increasing age. The GMC of IgG levels against serotype 14 was significantly higher than those against serotypes 1, 4, 3 and 7F in each age group ($p < 0.05$), except in the 2–4 years old group.

3.2. Pneumococcal serotype specific IgG antibody levels in IVIG preparations

Specific IgG levels against all serotypes included in PCV13 were measured by the ELISA protocol in 5 lots of each different IVIG preparation. The results are summarized in Fig. 2. All IVIG preparations contained relatively abundant IgG antibodies against all serotypes included in PCV13. There was no significant variation in IgG levels against all serotypes between individual lots of IVIG. However, there were significant differences in IgG levels to individual serotypes in each IVIG. IgG levels against serotypes 6A, 14 and 19A were significantly higher than those against serotypes 4, 7F and 9V in each IVIG preparation ($p < 0.05$). Comparing IgG levels to each serotype among the IVIG preparations, product B had consistently lower IgG levels than other products, with significantly lower levels of IgG against serotypes 3, 5, 6A, 14 and 19A than the other three products ($p < 0.05$).

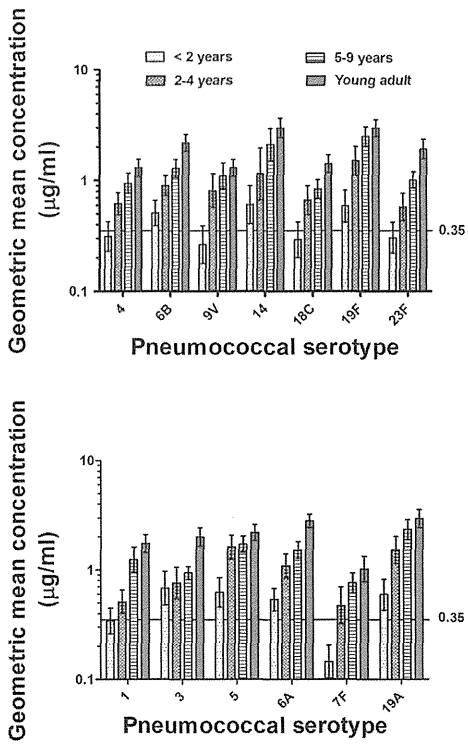

Fig. 1. Age-stratified geometric mean IgG concentrations (GMCs) with 95% confidence interval (CI). In the upper panel the PCV7 serotypes are presented and in the lower panel the other six serotypes are presented. The straight lines indicate the 0.35 μg/ml threshold concentration.

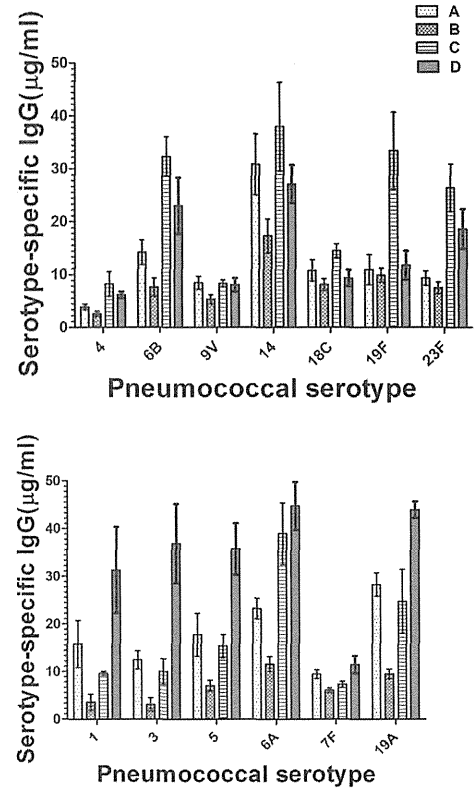

Fig. 2. The average serotype-specific IgG levels of five IVIG lots shown by bar graph with error bar of standard deviations. In the upper panel the PCV7 serotypes are presented and in the lower panel the other six serotypes are presented.

3.3. Opsonization index (OI)

MOPA for serotypes 6B and 19A was performed for 3 lots of each IVIG preparation. Table 1 shows the mean OIs of the three products. In general, each IVIG preparation had the ability to opsonize both serotypes 6B and 19A. Although product A did not have the highest levels of IgG against serotypes 6B and 19A when compared with the other three products, it had the highest OIs for both serotypes 6B and 19A. Product B had the lowest levels of IgG against serotype 19A of the four products; however, it had a higher OI than product C. The correlation coefficients couldn't be calculated because there

Table 1
Opsonophagocytic killing assay titers of polyclonal intravenous immunoglobulin preparations.

Product	Mean OI of 3 lots of each product	
	6B	19A
A	38058	1906
B	512	557
C	1305	254
D	5268	1123

The Ig concentration in the first dilution (1:4) was 12.5 mg/ml.
Capacity represents OI—maximum dilution of polyclonal intravenous immunoglobulin preparation at which 50% of the pneumococcal CFU were killed.
OI; opsonization index, CFU; colony-forming unit.

were only few sample data and their data didn't follow normal distribution.

4. Discussion

We have presented here data showing the levels of specific IgG against all serotypes included in PCV13, both in four IVIG preparations and in a healthy Japanese population. To our knowledge, this is the first report to have reported the levels of IgG against all PCV13 serotypes in both IVIG preparations and the Japanese population, using the WHO protocol.

In this study, infants aged <2 years had GMCs of <0.35 μg/ml against serotypes 1, 4, 7F, 9V, 18C and 23F and children ≥2 years old had GMCs of >0.35 μg/ml against all serotypes included in PCV13, even though they were non PCV7 immunized population. The age-stratified dynamics of the IgG concentrations were rather different to those reported in results from a similar study in the Netherlands [11]. We could not easily compare our results to theirs, because there were differences in sample numbers and they measured serotype-specific IgG concentrations by using a multiplex bead-based assay. Therefore, we compared the trend of our data to their data. Although they demonstrated that both infants aged <2 years and children aged 2–4 years had GMCs <0.35 μg/ml against most serotypes, our data showed that children aged 2–4 years had GMCs higher than the threshold. However, the incidence of IPD among infants aged <2 years was higher than that seen among other groups of children, both in the Netherlands [12] and Japan [13]. It is unclear why the incidence of IPD in Dutch children aged 2–4 years age group is low compared in Dutch <2 years, despite lower GMCs in this age group. Our results might suggest a possible correlation between the incidence of IPD and serotype-specific IgG levels when stratified by age group. The differences of serum GMCs between Dutch and Japanese might be caused by the differences of immune responses for *S. pneumoniae* in race and the degree of pneumococcal exposure in the regions. So far, there is no comparable data on the natural immune response of IPD between Dutch and Japanese population. However, according to the immunological data of recent clinical trials of PCV13 in two countries, the IgG levels after the immunization of PCV13 among both Japanese population and Dutch population increased more sufficiently than the IgG levels before the immunization of PCV13 [14, 15]. Therefore both Japanese and Dutch population is suggested to have good immunological responses for PCV13.

The development of natural immunity to pneumococci is likely due to exposure to carriers and/or to exposure to pneumococcal mucosal diseases such as otitis media, bronchitis or sinusitis. Other bacterial species may also induce cross-reacting antibodies to certain serotypes[16, 17]. Masuda et al. reported that all the children younger than 1 year carried *S. pneumoniae* and the carriage rate decreased with age in Japanese day-care centers [18]. We suggested that frequent exposure of pneumococci might cause to higher GMCs in Japanese children ≥2 years old. Most IPD pediatric patients are under 2 years old prior to introduction of PCVs.

We consider low pneumococcal serotype-specific IgG level is one risk factor of IPD. The routine immunization of infants prior to the exposure of pneumococci, exactly immunization from 2 month-old infants, with PCV may be effective for the prevention of IPD. In Japan, PCV7 was introduced as the voluntary immunization program from 2010. Japanese infant could be vaccinated PCV7 from April 2013 and finally can be vaccinated PCV13 from November 2013 as national immunization program. Therefore, it is suggested that most Japanese might have serotype-specific IgG titer against PCV13 serotypes (except PCV7 serotypes) which is about the same level as the results in this study.

Our results for the IgG levels in the IVIG preparations contrast with a similar study by Mikolajczyk et al. [6]. These authors showed antibody levels to serotypes 14 and 19F are normally several-fold higher than those to types 4, 6B and 9V. In our study, all of the IVIG preparations contained relatively high levels of IgG against serotype 6A, 14 and 19A. The difference might be explained by the variations in the age of donors, as well as differences in both the frequency of occurrence and the epidemiology of *S. pneumoniae* in these different countries.

Polyclonal IVIG has been shown to aid phagocytosis by coating bacteria with IgG and activating the complement cascade, or by producing opsonins by activation of the 'alternative complement pathway' [19]. Thus, specific antibody against *S. pneumoniae* may be critical. The minimum concentration of serotype-specific pneumococcal antibody required to be effective against IPD is unclear. We calculated a proposed dosage rate that would be effective against IPD by using the threshold of 0.35 μg/ml of specific antibody in serum. In Japan, the dose for severe infections such as sepsis and meningitis is 100–150 mg/kg IVIG with a concentration of 50 mg/ml as a single dose. If a 10-kg child was administered 150 mg/kg as a single dose, estimating a circulating blood volume of 800 ml, a 30 ml of the IVIG solution will be needed. Although the required serotype-specific IgG concentration against each serotype in an IVIG preparation would be 9.3 μg/ml (0.35 μg/ml × 800 ml divided by 30 ml) to achieve the threshold level in the child's blood, none of the IVIG preparations contain 9.3 μg/ml of serotype-specific IgG antibodies against serotype 4 and 9V. In particular, product B does not have the required IgG concentration against most serotypes, except serotypes 6A, 14, 19A and 19F. In a previous study, mortality has been significantly reduced among children with sepsis syndrome who received polyclonal IVIG at a dose of 400 mg/kg for 3 days [3]. If a 10-kg child is administered 400 mg/kg of IVIG solution which is volume of 80 ml as a one-time dose, serotype-specific IgG concentration in an IVIG preparation would only need to be 3.5 μg/ml (0.35 μg/ml × 800 ml divided by 80 ml) to achieve the threshold level. All IVIG preparations contain serotype-specific IgG against each PCV13 serotype at levels >3.5 μg/ml. It is suggested that patients with IPD, especially aged <2 years, might require sufficient dose of IVIG therapy because they have low serotype-specific IgG against most PCV13 serotypes. Therefore, we propose IVIG preparations would be more effective for patients with IPD if used at higher doses than the regular dose (100–150 mg/kg).

S. pneumoniae has other common structural components, such as Pneumococcal cell wall polysaccharide (C-polysaccharide), pneumococcal surface protein A (PspA), pneumococcal surface adhesion A (PsaA) and pneumolysin. Musher et al. measured mean IgG reactive against C-polysaccharide by ELISA in 15 healthy young adults and in 126 randomly selected hospital patients of all ages [20]. In three groups of patients (3 with acute purulent tracheobronchitis, 13 with non-bacteremic pneumococcal pneumonia, and 14 with *S. pneumoniae* bacteremia) at the time of admission, mean antibody levels were higher than the IgG levels in healthy adults. In the results, they suggested that naturally present anti-C-polysaccharide IgG did not protect against the evolution of acute pneumococcal infection from colonization to acute purulent bronchitis, from bronchitis to pneumonia, or from pneumonia to bacteremia. Holmlund et al. measured the antibody concentrations against PspA, PsaA and Ply by enzyme immunoassay in serum samples of 51 Filipino pregnant women, in six consecutive serum samples of 173 infants (samples from 7 to 48 weeks of age) and collected nasopharyngeal swabs from the infants [21]. The GMC of anti-PspA and -Ply decreased until 18 weeks of age and started to increase thereafter. The GMC of anti-PsaA in the infants increased significantly by age and reached the GMC of the mothers already at 14 weeks of age. High maternal anti-Ply antibodies were negatively

associated with the risk of pneumococcal carriage. According to these studies, IVIG preparation might contain specific IgG against these components, but, to our knowledge, there are no data about it. IVIG preparations will be suggested to have more effects for IPD patients, if specific IgG levels against their components in IVIGs would be measured.

There was no certain trend in our study between the pneumococcal ELISA and OI results, and similar results have been reported from another study [6]. The lack of correlation may be attributable to differences in antibody avidity or complement fixation activity, which were not examined in this study. In addition, the OI can have high intra-and/or inter-assay variability. While a functional test such as the OI is desirable, more research needs to be performed for assay optimization. Although there was the lack of correlation in the present study, these four IVIG preparations do have opsonophagocytic capacity against serotypes 6B and 19A. Although it might be necessary to measure OIs for other serotypes including PCV13, we couldn't measure them simultaneously because materials and labwares for MOPA were limited. Serotype 6B frequently causes IPD in Japan and IPD caused by serotype 19A is an increasing problem at present. Therefore we considered OIs of these serotypes were useful information. Further studies would be expected to evaluate opsonophagocytic activities of IVIG preparations.

In conclusion, we measured the IgG levels against PCV13 pneumococcal serotypes in both commercial IVIG preparations and the healthy non-PCV7 immunized Japanese population. The use of IVIG might be an adjuvant therapy for patients with IPD, especially for patients <2 years old. The results of our experiments support the concept that effective IgG levels for the treatment of IPD caused by all PCV13 serotypes would be achieved in human subjects after infusion of IVIG at an increased dose than the regular dosage used in Japan.

Conflict of interest

None.

Acknowledgments

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Protective properties of a fusion pneumococcal surface protein A (PspA) vaccine against pneumococcal challenge by five different PspA clades in mice

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ABSTRACT

An increase in the appearance of nonvaccine serotypes in both children and adults with invasive pneumococcal disease (IPD) after introduction of pneumococcal conjugate vaccine represents a limitation of this vaccine. In this study, we generated three recombinant pneumococcal surface protein A (PspA) proteins comprising PspA families 1 and 2, and we examined the reactivity of antisera raised in mice immunized with a PspA fusion protein in combination with CpG oligonucleotides plus aluminum hydroxide gel. The protective effects of immunization with PspA fusion proteins against pneumococcal challenge by strains with five different PspA clades were also examined in mice. Flow cytometry demonstrated that PspA3+2-induced antiserum showed the greatest binding of PspA-specific IgG to all five challenge strains with different clades. PspA2+4- or PspA2+5-induced antiserum showed the lowest binding of PspA-specific IgG to clade 3. Immunization with PspA3+2 afforded significant protection against pneumococcal challenge by five strains with different clades in mice, but immunization with PspA2+4 or PspA2+5 failed to protect mice from pneumococcal challenge by strains with clades 1 and 3. The binding of PspA-specific IgG in antisera raised by three PspA fusion proteins was examined in 68 clinical isolates from adult patients with IPD. Immunization of mice with PspA3+2-induced antiserum with a high binding capacity for clinical isolates expressing clades 1–4, but not clade 5. Our results suggest that the PspA3+2 vaccine has an advantage over the PspA2+4 or PspA2+5 vaccine in terms of a broad range of cross-reactivity with clinical isolates and cross-protection against pneumococcal challenge in mice.
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1. Introduction

Streptococcus pneumoniae is a major cause of morbidity and mortality caused by pneumonia, bacteremia, and meningitis worldwide [1]. After introduction of the seven-valent pneumococcal conjugate vaccine (PCV7) in children, significant declines in the incidence of invasive pneumococcal disease (IPD) caused by vaccine serotypes were reported in children and adults [2,3]. However, an increase in the incidence of IPD caused by non-PCV7 serotypes has been also observed in children and adults [3–5]. In addition, after introduction of a 13-valent pneumococcal conjugate vaccine (PCV13) in children, serotypes not included in PCV13 have been isolated with increasing frequency in pediatric and adult patients with IPD [6,7]. Because there are >90 different pneumococcal capsular serotypes, continuous supplementation of pneumococcal conjugate vaccines with new serotypes for serotype replacement may not be a practical strategy.

Previous studies have demonstrated that several pneumococcal proteins are potential vaccine candidates [8–11]. One candidate protein antigen is pneumococcal surface protein A (PspA), which is

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