study was the absence of a dyspnea index. Notwithstanding, the DSS correlated well with the D_{LCO} % predicted, less well with FVC % predicted and VC % predicted, and not with FEV_1/FVC. Although PAP is usually described as a restrictive lung disease, reductions in lung volumes in autoimmune PAP were minor and fell in the normal range in most patients, suggesting that these pulmonary function measures may be of limited usefulness in assessing the severity of PAP lung disease. Physiologically insignificant restriction is further supported by the absence of hypoventilation, even in severe cases. Thus, in autoimmune PAP, hypoxemia is primarily due to reduced oxygen diffusion and possibly ventilation–perfusion mismatching.

Infections were less common among Japanese PAP registrants than previously reported (2). Furthermore, although *Nocardia* was identified in 60% of reported PAP cases complicated by infection (2), no cases of *Nocardia* infection were observed in our study during the period of observation. It is possible that these differences represent reporting bias or differences in clinical care of early infections because a number of the prior reports reflect infectious complications occurring over four decades.

Our observation that GM-CSF autoantibody levels did not correlate with disease severity as measured by the presence of symptoms, pulmonary function testing, or the DSS is consistent with prior reports (2, 3, 22). Because GM-CSF autoantibodies in patients with PAP are polyclonal, it is possible that measuring the level of neutralizing antibody may provide a better correlation with disease severity. We have reported a patient with autoimmune PAP in whom serial measurement of serum GM-CSF neutralizing activity correlated well with disease severity (47). Furthermore, the serum GM-CSF neutralizing activity was reduced in a patient who was successfully treated with inhaled GM-CSF (47). Pulmonary compartmentalization of GM-CSF antibodies may be important in determining disease severity and could explain the lack of correlation with serum autoantibody levels. Neither the autoantibody levels, proportion of symptomatic individuals, pulmonary functions, nor DSS correlated with the duration of disease, which is consistent with the concept that disease severity does not worsen with time in most patients.

The method used to measure GM-CSF autoantibody levels is similar to prior reports (17) except that a new monoclonal GM-CSF autoantibody standard was used. Although this standard yields reproducible results, it yields autoantibody levels one seventh that of the previous GM-CSF autoantibody standard isolated from pooled PAP serum (3, 15, 17, 19) and similar in sensitivity and specificity. Our results support the use of GM-CSF autoantibody measurement in the diagnosis of autoimmune PAP as an adjunct to chest CT and bronchoscopy.

Conflict of Interest Statement: None of the authors has a financial relationship with a commercial entity that has an interest in the subject of this manuscript.

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APPENDIX

The patients were referred to one of the nine primary clinical research centers by the following physicians (only one individual is listed per institution because of space limitations): Arata Azuma (Nippon Medical School), Masato Katagiri (Kitazato University), Masafumi Niijima (Narita Red Cross Hospital), Shinobu Akagawa (NHO Tokyo Hospital), Masayuki Nara (Tohoku University), Akira Fujita (Metropolitan Fuchu Hospital), Ryo Takahashi (Osaka Prefectural Medical Center for Respiratory and Allergic Diseases), Jun Sato (Hamamatsu University School of Medicine), Hidenori Ichiyasu (Kumamoto University), Yoshihiro Honda (Sendai Kohsei Hospital), Yoshio Taguchi (Tenri Hospital), Masakazu Aitani (NTT West Osaka Hospital), Masanori Nakanishi (NHO Tsuruga Hospital), Tetsuo Yamaguchi (JR Tokyo General Hospital), Muneharu Maruyama (Toyama University Hospital), Atsuhiro Fujii (Juntendo University), Kohei Yamauchi (Iwate Medical University), Towako Nagata (Nagasaki University), Tatsuro Mikawa (Yodogawa Christian Hospital), Toshihiko Hashizume (Yokohama Kyosai Hospital), Sakae Honnma (Toranomon Hospital), Masato Tohyama (University of the Ryukyus), Masaharu Nagayama (Shizuoka City Hospital), Noriharu Shijubo (Sapporo Medical University), Koichiro Takahashi (Saga Medical University), Iwao Komuro (Metropolitan Hiroo Hospital), Mihoko Doi (Hiroshima University), Kaoru Maki (Matsue Seikyo Hospital) Yoshitsune Sando (Gunnma University), Hiroo Miyazaki (Fukuroi Municipal Hospital), Youkou Shibata (Yamagata University), Hirohisa Toga (Kanazawa Medical College), Naotoshi Suruta (NHO Wakayama Hospital), Hiroaki Kume (Nagoya University), Ken Nawa (Hitachi General Hospital), Kaneo Kawazoe (Naka Tsushima Hospital), Watako Takehara (Chubu Rosai Hospital), Yasuhiro Ieda (Kinki University Sakai Hospital), Masaru Yauchi (Ishimaki Red Cross Hospital), Yuji Akiba (Asahikawa Kosei Hospital), Masako Toh (The Fraternity Memorial Hospital), Toshiyuki Yamauchi (Keihai Rosai Hospital), Yuzuru Inoue (Shin Yamate Hospital), Kenji Kohno (Kyoto Prefectural University of Medicine), Machiko Arita (Kurashiki Central Hospital), Kazunari Himeno (Fujita Health University), Nobuto Kishimoto (Takamatsu Municipal Hospital), Masaya Yamasato (NHO Minami Yokohama Hospital), Aya Sugawara (Fukishima Medical University), Atuko Kobayashi (Saiseikai Suita Hospital), Katsunori Sugisaki (Oita University), Kenichiro Ohtani (Osaka City University Medical School), Yoshikazu Ishii (Jichi Medical University), Yoshiki Kobayashi (Takatsuki Red Cross Hospital), Shigeru Koyama (Nagano Red Cross Hospital), Hiroko Kimura (Tohoku Rosai Hospital), Atuhiro Goto (Okazaki City Hospital), Amihiko Hirano (Wakayama Medical University), Jun Shiraki (Kochi Health Science Center), Fumiko Sugatani (Teine Kijinkai Hospital), Akira Miyashita (Yokohama City University School of Medicine), Momoyo Ukai (Tokushima University), Yoshida Makino (Osaka Medical College), Hidenori Mori (Gifu University), Susumu Oguri (NHO Minami Kyoto Hospital), Taku Inoue (Sano Kousei Sougou Hospital), Masaaki Takahashi (Asahikawa Medical University), Michihiro Yoshimi (Kyusyu University), Toshiaki Hidaka (Koga Sogo Hospital), Masahiko Iwaoka (Fujieda Municipal Central Hospital), Daizen Cho (Tsubame Rosai Hospital), Eishi Ito (Hakodate City Hospital), Hiroyo Okurakata (Saiseikai Sanjo Hospital), Hiroshi Saiki (NHO Miyazaki Hospital), Jun Katsuta (Takayama Red Cross Hospital), Yoshifumi Imazu (Miyazaki University), Mikiko Ono (Kagoshima University), Tatsuya Hosono (Jichi Medical College), Shinji Takeuchi (Takamatsu Red Cross Hospital), Kenji Konishi (Seirei Hamamatsu General Hospital), Mikio Oka (Kawasaki Medical University), Takefumi Saito

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Original Article

Drug-resistant pneumococci in children with acute lower respiratory infections in Vietnam

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Abstract ***Background***: Acute lower respiratory infections (ALRI), primarily pneumonia, are the leading cause of death in children under 5 years of age. Most of these deaths occur in Africa and southeast Asia. Increasing rates of drug resistance in pneumococcal strains emphasize the necessity of prevention of pneumococcal vaccines. The aim of the present study was to determine the frequency of drug resistance and the distribution of serotype of pneumococcal strains isolated from pediatric patients with ALRI in Vietnam.

Methods: Two hundred and twenty pediatric patients with ALRI under 5 years of age were enrolled in Hanoi, Vietnam between 2001 and 2002. Bacterial pathogens with a heavy growth (10^6 c.f.u./mL) were isolated from nasopharyngeal secretions on quantitative culture. Fifty-three pneumococcal strains isolated from the nasopharynx of pediatric patients were examined for antibiotic susceptibility including drug-resistant genes and serotyping.

Results: A total of 73.6% of pneumococcal strains were genotypic penicillin-resistant *Streptococcus pnemoniae* (gPRSP), possessing altered penicillin-binding protein genes *pbp 1a + 2x + 2b*; 67.9% of these strains were gPRSP and simultaneously had the *ermB* gene, which is responsible for high resistance to erythromycin. The majority of gPRSP strains were serotype 19F or 23F.

Conclusion: gPRSP strains with serotype 19F or 23F are highly prevalent among pediatric patients with ALRI under 5 years of age in Hanoi, Vietnam.

Key words acute lower respiratory infection, children, drug resistance, serotyping, *Streptococcus pneumoniae*.

Acute lower respiratory infections (ALRI), primarily pneumonia, are the leading cause of death in children under 5 years of age, but diagnosis is difficult both in industrialized and developing countries. A recent report indicated that 1.9 million children died from ALRI in 2000, worldwide, and that 70% of these deaths occurred in Africa and southeast Asia.[1] The two leading bacterial pathogens of pneumonia are *Streptococcus pneumoniae* and *Haemophilus influenzae*.[2,3]

An increasing prevalence of pneumococcal strains resistant to β-lactams has been observed in both developing and developed countries during the last decade.[4] Recent studies have also found high frequencies of penicillin- and macrolide-resistant pneumococci as a respiratory pathogen in several Asian countries, including Japan.[5,6] While the resistance of *S. pneumoniae* to β-lactams has been shown to be associated with mosaic mutations in the penicillin-binding protein genes *pbp 1a*, *pbp 2b* and *pbp 2x*,[7,8] macrolide resistance is mediated by methylation of the 23S rRNA methylase encoded by the *ermB* gene and macrolide efflux via the *mefA* gene.[9,10]

In children, bacterial pneumonia is generally preceded by asymptomatic bacterial colonization. It is well understood that bacterial colonization plays a central role in bacterial pneumonia.[11] Previous studies used nasopharyngeal swab samples to examine antibiotic resistance and serotyping for treatment and vaccine formulation among children.[12–15] Increasing rates of drug resistance in pneumococcal strains emphasize the necessity of prevention of pneumococcal vaccines. Although two previous studies have recently reported that the pneumococcal conjugate vaccine is effective in reducing the incidence of pneumonia as well as invasive pneumococcal pneumonia among African infants,[16,17] few data on the drug resistance or serotype distribution of penumococcal strains isolated from pediatric patients with ALRI are currently available in developing countries in Southeast Asia. The aim of the present study was therefore to examine antibiotic susceptibility, including drug-resistant genes and serotyping of pneumococcal strains isolated from the nasopharynx of patients with ALRI, among children in Hanoi, Vietnam.

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Methods

Patient population and study design

Two hundred and twenty pediatric patients with ALRI were enrolled at the National Pediatric Hospital (NPH), a 600-bed hospital, or the Bach Mai Hospital (BMH), a 1400-bed hospital, the two tertiary hospitals in Hanoi, Vietnam, between January 2001 and December 2002. The distance between the two hospitals is 6 km, and each hospital covers Hanoi and its suburban area. The criteria for inclusion were: (i) age under 5 years and a diagnosis of ALRI made within 24 h of admission; (ii) clinical symptoms of a productive cough, fast breathing and fever >37.5°C; and (iii) crackle in the lung fields on auscultation. Exclusion criteria were: (i) age above 5 years; (ii) illness of non-infectious etiology; (iii) failure to provide consent. The present study was approved by the Institutional Review Boards of National Institute of Hygiene and Epidemiology, and a signed consent form was obtained from each subject.

Bacteriological examinations

A previous study reported the isolation of bacterial pathogens on quantitative culture using oropharyngeal swab samples from pediatric patients.[18] Because nasopharyngeal samples are regarded to be superior to oropharyngeal samples for the detection of the two leading pathogens (*S. pneumoniae* and *H. influenzae*),[19] we examined nasopharyngeal secretions from 220 patients with ALRI using two flexible swabs (Medical Wire & Equipment, Wiltshire, England, UK) for quantitative bacterial culture. After weighing the nasopharyngeal swab samples on a microbalance, the volume of the sample was determined to be approximately 0.01 mL. This sample was diluted in brain–heart infusion broth (BBL, Becton Dickinson, Cockeysville, MD, USA), and a 10-fold dilution was then prepared in saline as described previously.[18,20] A quantitative bacterial culture was carried out on trypticase soy agar (BBL, Becton Dickinson) containing 7% defibrinated rabbit blood and incubated in a 5% CO_2 incubator at 37°C overnight. Possible bacterial pathogen with a heavy growth (10^6 c.f.u./mL) was isolated.[21]

Fifty-three strains of *S. pneumoniae* isolated from 220 pediatric patients with ALRI were investigated for antibiotic susceptibility including analysis of drug-resistance genes and serotyping. Forty isolates were from NPH and 13 isolates were from BMH, respectively. The minimum inhibitory concentrations (MIC) were determined using agar dilution (National Committee for Clinical Laboratory Standards 2001),[21] and serotyping was done on the basis of the quellung reaction with capsular antisera (Statens Serum Institut, Copenhagen, Denmark). Three penicillin-binding protein genes (*pbp1a, pbp2x, pbp2b*) only in susceptible strains and macrolide-resistant genes (*mefA* and *ermB*) were amplified on polymerase chain reaction (PCR; Wakunaga Pharmaceutical, Hiroshima, Japan) according to the manufacturer's instructions.[7,22]

Results

The 50% MIC (mic_{50}) and 90% MIC (mic_{90}) (μg/mL) against the 53 strains of *S. pneumoniae* are listed in Table 1. The MIC for penicillin G and ceftriaxone against 53 pneumococcal isolates (one strain per patient) based on PCR results are shown in Figure 1. The *in vitro* activity of penicillin G against these strains indicated that 34 patients were associated with penicillin-resistant strains (MIC, 2 μg/mL in 20 strains; 4 μg/mL in 14 strains, penicillin G; Fig. 1a). The grade of resistance was much milder for a third-generation cephem, ceftriaxone, than for penicillin G (Fig. 1b). According to a new breakpoint for ceftriaxone,[23] 50 (94.3%) of the 53 strains were regarded as susceptible. Interestingly, of the 53 strains, 39 (73.6%) were genotypic penicillin-resistant *S. pnemoniae* (gPRSP), possessing altered *pbp 1a + 2x + 2b* genes (Table 2).[24] Eight strains possessed altered *pbp 2x + 2b* genes. Lack of mutation was found in only six strains. These pneumococcal strains were also frequently associated with macrolide-resistant genes, such as *mefA and ermB*. Twenty-one strains were found for both *mefA* and *ermB*, 21 strains for *ermB* alone, and four strains for *mefA* alone, and seven strains for no mutation. Thirty-six pneumococcal strains (67.9%), therefore, were gPRSP and simultaneously had the *ermB* gene, which is responsible for high resistance to erythromycin. Relationship between the genotype patterns of the *pbp* gene (Table 3) or the macrolide-resistant gene and serotype distribution among 53 isolates are shown in Table 4). The most frequently observed serotypes were 19F (47.2%), 23F (32.1%) and 6B (5.7%). The rate of coverage of 7-valent conjugate vaccine was 88.7%. Non-covered serotypes in these strains were 6B (three strains), 9L (two strains) and others. More importantly, 92.3% (36/39) of gPRSP and 94.4% (34/36) of gPRSP associated with *ermB* gene were serotype 19F or 23F. We also compared the frequency of drug-resistant genes and the serotype distribution between 40 isolates from NPH and 13 isolates from BMH. Although six strains with no *pbp* gene mutation were found only in isolates from NPH, no difference was found in the frequency of *pbp* genes including gPRSP or macrolide-resistant genes and in the frequency of major serotypes, such as 19F and 23F, between pneumococcal isolates from the two hospitals (data not shown).

Table 1 MIC of 53 strains of *Streptococcus pneumoniae*

Antibiotic	MIC (μg/mL)		
	Range	50%	90%
Penicillin G	0.01–4.00	2	4
Ampicillin	0.01–16.00	4	8
Cefuroxime	0.02–16.00	4	8
Ceftriaxone	0.01–4.00	1	1
Erythromycin	0.01–128	64	128
Minocycline	0.032–16.00	8	8
Gentamicin	2.00–16.00	8	8
Chloramphenicol	1.00–16.00	4	8
Ofloxacin	0.50–4.00	2	4
Co-trimoxazole	2.00–128	128	128

MIC, minimum inhibitory concentration.

Discussion

The present findings indicate that *S. pneumoniae* isolated from pediatric patients with ALRI were highly resistant to penicillin, erythromycin and co-trimoxazole. A similar finding of antibiotic resistance to these drugs was found for *H. influenzae* strains isolated from these patients.[25] An unique finding in the present

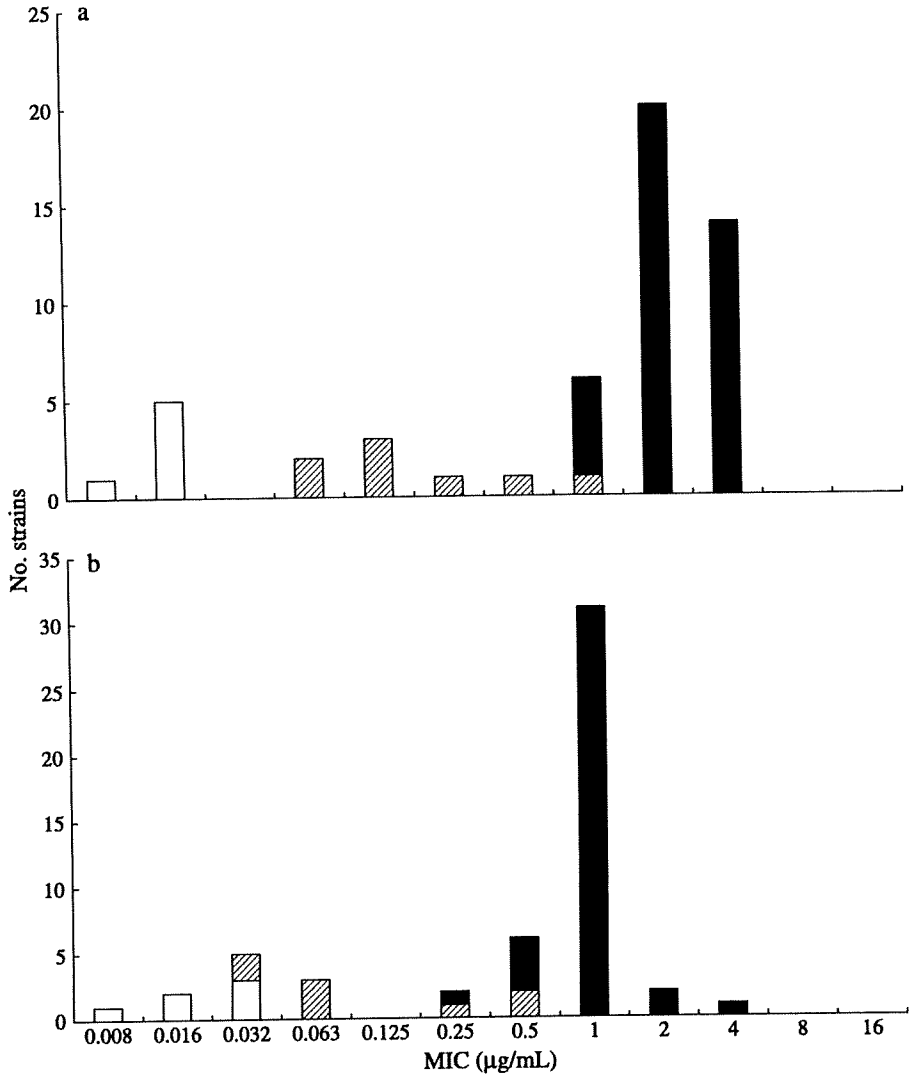

Fig. 1 Relationship between minimum inhibitory concentration (MIC) for two β-lactam antibiotics, (a) penicillin G and (b) ceftriaxone, and genotype of the penicillin-binding protein (*pbp*) genes in 53 clinical isolates of *Streptococcus pnemoniae* from pediatric patients with acute lower respiratory infections. (□) No mutation; (▨) *pbp 2x + 2b* ; (■) *pbp 1a + 2x + 2b.*

study is that most of the pneumococcal isolates from pediatric patients with ALRI were gPRSP with macrolide-resistant genes, which are associated with serotype 19F or 23F. A previous study reported that two international multidrug-resistant clones Spain 23F-1 and Taiwan 19F-14 were spreading among children with upper respiratory tract infections in Hanoi, Vietnam.[26] Another study on surveillance of drug-resistant pneumococcal strains also found the spread of Spain 23F-1 clone among patients with invasive pneumococcal infections in Ho Chi Minh City, Vietnam.[27] The frequency of serotype 19F or 23F (79.3%) in pediatric patients with ALRI in the present study was much higher than that of pediatric patients with upper respiratory tract infections (53%)[26] or invasive pneumococcal infections (43.3%) in Vietnam.[27] The most frequently observed serotype was 23F, but not 19F, in those studies. The present data, therefore, suggest a recent increase of serotype 19F multidrug-resistant pneumococcal strains in pediatric patients with ALRI in Hanoi, Vietnam.

Table 2 Genotype of drug-resistant genes and MIC in 53 strains of *Streptococcus pneumoniae*

Genotype	*n* (%)	MIC range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)
			Penicillin	
No mutation	6 (11.3)	0.01–0.02	0.02	0.02
pbp 2x + 2b	8 (15.1)	0.01–1.0	0.13	1
pbp 1a + 2x + 2b	39 (73.6)	1.0–4.0	2	4
			Erythromycin	
No mutation	7 (13.2)	0.01–0.06	0.03	0.06
MefA	4 (7.5)	0.5–4.0	1	4
ErmB	21 (39.6)	1.0–128	32	128
mefA + ermB	21 (39.6)	4.0–128	128	128

MIC, minimum inhibitory concentration.

The high resistance of the two major pathogens (*S. pneumoniae* and *H. influenzae*) to co-trimoxazole in the present study and in

Table 3 *pbp* genotype and serotype among 53 strains of *Streptococcus pneumoniae*

Genotyping	No. isolates with serotype									
	19F	23F	6B	9L	14	19B	9A	17F	Non-typeable	Total
No mutation	3			2				1		6
pbp 2x + 2b	1	2	3		2					8
pbp1a + 2x + 2b	21	15				1	1		1	39
Total	25	17	3	2	2	1	1	1	1	53

pbp, penicillin-binding proteins.

Table 4 Genotype of macrolide-resistant genes and serotype among 53 strains of *Streptococcus pneumoniae*

Genotyping	No. isolates with serotype									
	19F	23F	6B	9L	14	19B	9A	17F	Non-typeable	Total
No mutation	2	2		2				1		7
mef	1	1			1				1	4
ermB	5	12	3		1					21
mefE + ermB	17	2				1	1			21
Total	25	17	3	2	2	1	1	1	1	53

our previous report may facilitate attempts to use penicillin as an antibiotic for treating pneumonia among children.[25] Although treatment with oral amoxicillin (45 mg/kg) has been shown to be equally effective to that of injectable penicillin for severe pneumonia among children in developing countries,[28] it might be recommended to give a high dose of oral amoxicillin or injectable penicillin or third-generation cephem for children with pneumonia because of the high prevalence of gPRSP or TEM-1 type β-lactamase-producing *H. influenzae* among children in Hanoi, Vietnam.[25] A recent study also reported that drug resistance of nasal carriage isolates of *S. pneumoniae* was increasing in rural areas as well as in urban areas in Vietnam.[29] To prevent the progress of antibiotic-resistant respiratory pathogens, the use of antibiotics without a prescription should be limited in Vietnam.[13]

More importantly, the early introduction of pneumococcal conjugate vaccine is strongly recommended in Vietnam, because the coverage of serotypes by a 7-valent conjugate vaccine was high (88.7%) in pediatric patients with ALRI in the present study. Furthermore, although 7-valent or 9-valent pneumococcal conjugate vaccine are currently available, a new formulation of pneumococcal conjugate vaccine with two serotypes, such as 19F and 23F, may be a possible strategy for reducing the cost of this vaccine in Vietnam.

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Additive effect of pneumococcal vaccine and influenza vaccine on acute exacerbation in patients with chronic lung disease

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ABSTRACT

To determine the clinical efficacy of combined vaccination with 23-valent pneumococcal vaccine (PV) and influenza vaccine (IV) against pneumonia and acute exacerbation of chronic lung diseases (CLD), we conducted an open-label, randomized, controlled study among 167 adults with CLD over a 2-year period. Subjects were randomly assigned to a PV + IV group ($n = 87$) or an IV group ($n = 80$). The number of patients with CLD experiencing infectious acute exacerbation ($P = 0.022$), but not pneumonia ($P = 0.284$), was significantly lower in the PV + IV group compared with the IV group. When these subjects were divided into subgroups, an additive effect of PV with IV in preventing infectious acute exacerbation was significant only in patients with chronic obstructive pulmonary diseases ($P = 0.037$). In patients with CLD, the Kaplan–Meier survival curves demonstrated a significant difference for infectious acute exacerbation ($P = 0.016$) between the two groups. An additive effect of PV with IV on infectious acute exacerbation was found during the first year after vaccination ($P = 0.019$), but not during the second year ($P = 0.342$), and was associated with serotype-specific immune response in sera of these patients who used PV during the same period.

1. Introduction

Acute exacerbation frequently occurs among patients with chronic lung diseases (CLD), such as chronic obstructive pulmonary disease (COPD) and sequelae of pulmonary tuberculosis (SPTB) [1,2]. Morbidity, mortality and health-care costs of these patients largely result from on acute exacerbations [3]. Acute exacerbations are primarily triggered by bacterial or viral pathogens in COPD and SPTB. While *Streptococcus pneumoniae* (*S. pneumoniae*) is the most commonly identified cause of community-acquired pneumonia (CAP) by accounting for 16.5–38.9% of CAP among adults [4,5], this pathogen is also responsible for 8–25% of acute exacerbation in patients with CLD or COPD, which makes it a major bacterial pathogen [2,6,7]. Viral pathogens are also capable of inducing acute exacerbation of COPD, and the influenza virus was frequently detected in 5–29% of exacerbation of COPD [8,9].

Since antibodies to pneumococcal capsular polysaccharide (PPS) and complement provide protection against *S. pneumoniae* with homologous or cross-reactive capsular serotypes [10], pnemococcal polysaccharide vaccine (PV) is effective for preventing invasive pneumococcal diseases in patients with chronic illness, such as CLD. PV is, therefore, recommended for these patients [11–13]. Although the previous studies reported that PV is not effective in preventing pneumonia or acute exacerbation in patients with COPD [14–16], a recent, prospective study demonstrated an effect of PV in preventing pneumonia in such patients with less than 65 years of age with severe airflow obstruction [17]. In addition, a retrospective study previously reported the additive effects of PV with influenza vaccine (IV) in the reduction of hospitalization

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stays and death among elderly persons with CLD [18,19]. Although a large-scale prospective study demonstrated the additive effects of PV and IV in reducing hospital mortality due to pneumonia among elderly persons [20], no prospective study has been conducted to find the additive effects of PV combined IV for preventing pneumonia or acute exacerbation in patients with CLD. This open-label, randomized, controlled study was designed to determine whether PV and IV combined are superior to IV alone in preventing pneumonia or acute exacerbation among patients with CLD.

2. Materials and methods

2.1. Study design

For this study, 191 patients with CLD in a stable condition were enrolled after providing written informed consent at the respiratory clinic of 13 hospitals in the district of Kyushu and Okinawa, Japan between November 2001 and April 2002. All potentially eligible subjects (at least twice as many as the enrolled cases) were contacted by the members of the Pneumococcal Vaccine Trialist Group, belonging to one of these hospitals. As the study investigators, these doctors had a role in selecting the subjects for the study enrollment. Inclusion criteria were patients with CLD who previously experienced acute exacerbations, were able to comply with a schedule of monthly clinical visits and were between 40 and 80 years of age. Patients who were pregnant or had immunocompromised conditions such as active malignant diseases, renal insufficiency in dialysis or HIV infection, hypogammaglobulinemia or anatomical or functional asplenia and who had previously received 23-valent PV (Pneumovax, Banyu, Japan) were excluded. The enrollees were randomly assigned in equal proportion to either the group receiving PV and IV (The Chemo-Sero-Therapeutic Research Institute, Kumamoto, Japan) or IV alone. By sealed envelope, each study site was randomly allocated either 10 or 20 cases. Equal numbers of sealed envelopes each containing a card indicating either the PV + IV group or the IV group were prepared, and each study participant chose an envelope. This vaccine allocation was done by the study investigators. In addition, the doctors who screened the subjects also conducted the exclusions and enrollments.

While the participants in the PV + IV group were separately immunized with 0.5 ml of PV and 0.5 ml of IV on separate occasions in 1 month intervals, the participants in the IV group were immunized with 0.5 ml of IV alone. All participants received IV once in both the 2001/2002 and 2002/2003 seasons. For this study, our group used a trivalent, split-virion, influenza vaccine, containing A/NewCaledonia/20/99H1N1, A/Panama/2007/99H3N2, and B/Johannesburg/5/99 for the 2001/2002 season; and for the 2002/2003 season, the study was conducted using vaccine containing A/NewCaledonia/20/99H1N1, A/Panama/2007/99H3N2 and B/Guangdong/7/97.

Demographic data were obtained from each participant at the time of enrollment. All participants were examined, typically once a month, at each hospital by physicians who were the members of the Pneumococcal Vaccine Trialist Group at each study hospital in the Kyushu and Okinawa districts. Patients were also asked to visit each study hospital for examination by a study physician, if they developed a fever, cough and sputum, or experienced breathlessness during the 2-year follow-up period.

To monitor the concentrations of anti-PPS IgG, serum samples were collected from the patients of the PV + IV group immediately before and at 1 month, 6 months, 1 year, and 2 years after the initial pneumococcal vaccination. Separated sera were stored frozen at −80 °C until used. All studies described herein were approved by the institutional review board of each institution.

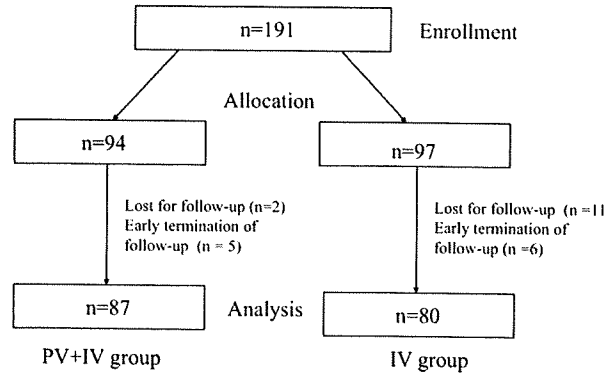

Fig. 1. Flow diagram of study patients with chronic lung disease.

2.2. Study population

One hundred and ninety-one patients with CLD were assigned to either the PV + IV group ($n = 94$) or to the IV group ($n = 97$) (Fig. 1). During 2-year follow-up period, 2 and 11 subjects were lost from the PV + IV group and IV groups, respectively. In addition, an early termination of follow-up occurred for 5 subjects for the PV + IV group and for 6 subjects in the IV group because they wanted to withdraw from the study. Subsequently, 87 subjects in the PV + IV group and 80 subjects in the IV group completed the analysis.

2.3. Outcome measures

The outcome measured was recorded as either the time to the first episode of pneumonia or to acute exacerbation after the enrollment in this study. A pneumonia diagnosis was based on either clinical symptoms (cough, sputum or fever) plus increased white blood cell counts or serum C-reactive protein, and the appearance of a new infiltration on a chest radiograph [21]. Acute exacerbation in CLD was defined by criteria as previously described [22]: (1) increased dyspnea, (2) increased sputum volume and (3) increased sputum purulence, and (4) absence of newly appeared infiltration on a chest radiograph. Acute exacerbation was diagnosed when two of the three respiratory symptoms existed or when one of these and one of additional symptoms, such as a fever without any other causes or increased cough was present [23]. When the laboratory examinations revealed an increase in their white blood cell counts or serum C-reactive protein, in addition to the clinical symptoms of acute exacerbation, patients were diagnosed as infectious acute exacerbation, and were therefore classified into one of two categories: either infectious or non-infectious acute exacerbation. Furthermore, when *S. pneumoniae* was isolated from purulent sputum in cases of acute exacerbation, patients were diagnosed as pneumococcal acute exacerbation as a subcategory of infectious acute exacerbation.

2.4. Measurement of anti-PPS IgG

Serum samples for serotype-specific IgG were available from only 35 of 87 patients with CLD in the PV + IV group for all intervals—1 month, 6 months, 1 year, and 2 years. 7 subjects died during the 2-year period, and 45 of the remaining subjects lacked at least one serum sample from the time interval at either 6 months, 1 year or 2 years. The concentrations of serotype-specific IgG were measured as previously described elsewhere [24]. The US reference

pneumococcal antiserum (89-SF), courtesy of Dr. Carl Frasch, was adsorbed to CWPS, but all other samples were adsorbed to CWPS (5 μg/ml) and 22F PPS (10 μg/ml) [25]. Serotype-specific IgG was determined for the four serotypes (6B, 14, 19F and 23F) that are the most prevalent among adult patients with pneumococcal infections in the US and Japan [5,26].

2.5. Statistical analysis

The case numbers of patients with COPD, SPTB, and other CLDs, and the case number of patients experiencing pneumonia, infectious acute exacerbation, pneumococcal acute exacerbation, and non-infectious acute exacerbation and the case numbers of death were compared using a χ^2-test. A Kaplan–Meier estimator was used to calculate the survival curve for subjects who developed neither pneumonia nor infectious or non-infectious acute exacerbation during the 2-year study period. The Cox's proportional hazard model was used to evaluate the effect of PV on the incidence of pneumonia and infectious or non-infectious exacerbation both in the first and second 1-year. The effects of age (age older than 55 years, 65 years, and older than 75 years) and female sex were considered in multivariate analysis. Differences in geometric mean concentrations (GMCs) of serotype-specific IgG over time were assessed using the Wilcoxon signed-ranks test. An interim target sample size of 82 was chosen to ensure that there would be at least an 80% chance to detect a difference of 0.2 (0.2 vs. 0.4) episodes per person per year, with a one-sided alpha level of 0.05, in the frequency of admission due to pneumonia between groups of PV + IV and IV. Data was considered to be statistically significant, if the P values were less than 0.05.

3. Results

Ages (mean ± S.D. years) and male sex (%) were 69.0 ± 9.0 and 63.5 for total subjects, 67.8 ± 9.5 and 69.0 for the PV + IV group, and 70.1 ± 7.4 and 57.5 for the IV group. The numbers of patients with three subcategories of CLD (COPD, SPTB and other CLDs) in the PV + IV and the IV groups are shown in Table 1. Other CLDs were bronchiectasis (n = 20; 10 for the PV + IV group and 10 for the IV group), bronchial asthma (n = 13, 6 for the PV + IV group and 7 for the IV group), pneumoconiosis (n = 14, 7 for the PV + IV group and 7 for the IV group), interstitial pneumonia (n = 9, 3 for the PV + IV group and 6 for the IV group), diffuse panbronchiolitis (n = 5, 4 for the PV + IV group and 1 for the IV group), and sarcoidosis (n = 1 for the IV group). Fifty-nine subjects received home oxygen therapy (HOT) (27 for the PV + IV and 32 for the IV), and 11 subjects (6 for the PV + IV and 5 for the IV) were treated with noninvasive positive pressure ventilation (NPPV). While a significant difference was found in the number of patients with SPTB between the two groups, no significant difference was found in the number of patients with COPD or other CLDs, nor in the number of patients receiving either HOT or NPPV.

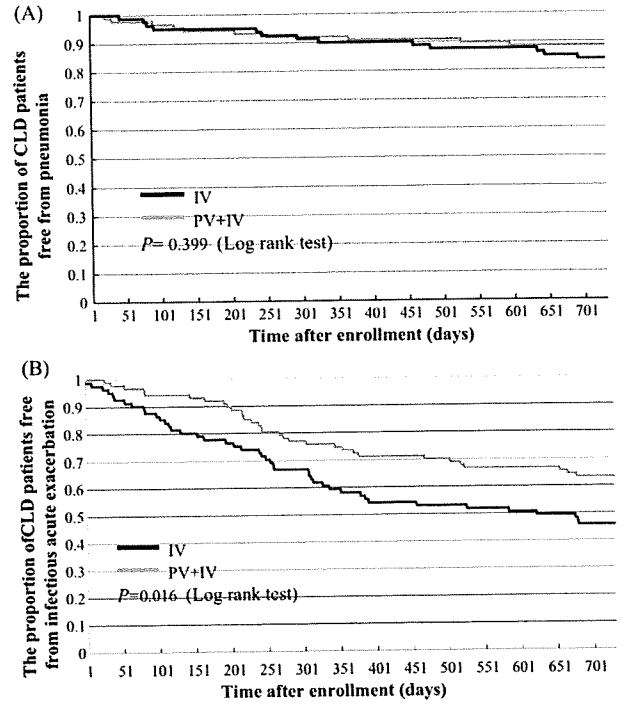

Fig. 2. Kaplan–Meier survival curves for patients with chronic lung diseases (CLD) showing the proportion of subjects free from pneumonia (A) and infectious acute exacerbation (B) between PV + IV group and IV group during the follow-up period.

The incidence of pneumonia (0.096 episodes/(patient year)) with CLD in this study was twice as higher as those of previous cohort studies of COPD patients (0.047–0.055 episodes/(patient year)) [14,17]. No significant difference was found in the number of patients developing pneumonia between the PV + IV group and the IV group (Table 1). Similarly, no significant difference was found in the Kaplan–Meier survival curves for pneumonia between the two groups (P = 0.399, Fig. 2A).

Table 1
Demographic features and outcome of patients with chronic lung diseases (CLD)

Characteristics	No. of patients (%)			P-value
	All subjects (n = 167)	PV + IV group (n = 87)	IV group (n = 80)	
Subcategories of CLD				
COPD	55(32.9)	24(27.6)	31(38.8)	0.125
Sequelae of pulmonary tuberculosis	50(30.0)	33(37.9)	17(21.3)	0.018
Other CLDs	62(37.1)	30(34.5)	32(40.0)	0.461
Outcome				
Pneumonia	25(32.9)	13(27.6)	12(38.8)	0.284
Acute exacerbation				
Infectious	76(45.5)	32(36.8)	44(55.0)	0.022
Pneumococcal	6(3.6)	1(1.1)	5(6.3)	0.106
Non-infectious	15(9.0)	9(10.3)	6(7.5)	0.557
Death	14(8.4)	7(8.0)	7(8.8)	0.870

PV: pneumococcal polysaccharide vaccine, IV: influenza vaccine, COPD: chronic obstructive pulmonary disease. Acute exacerbations are classified into two categories: infectious and non-infectious acute exacerbation. Infectious acute exacerbation involves pneumococcal acute exacerbation.

Table 2
Outcome of patients with COPD, sequelae of pulmonary tuberculosis (SPTB) and other chronic lung diseases (CLDs) **by vaccine group** during 2 years after vaccination

Subcategories of CLD/outcome	No. of patients (%)		*P*-value
	PV + IV group (n = 87)	IV group (n = 80)	
COPD			
Pneumonia	6 (6.9)	5 (6.3)	0.615
Acute exacerbation			
Infectious	9 (10.3)	21 (26.3)	0.037
Non-infectious	5 (5.7)	2 (2.5)	0.315
SPTB			
Pneumonia	3 (3.4)	2 (2.5)	0.218
Acute exacerbation			
Infectious	10 (11.5)	7 (8.7)	0.442
Non-infectious	2 (2.3)	3 (3.8)	0.594
Other CLDs			
Pneumonia	4 (4.6)	5 (6.3)	0.379
Acute exacerbation			
Infectious	13 (14.9)	16 (20.0)	0.599
Non-infectious	2 (2.3)	1 (1.3)	0.616

PV: pneumococcal polysaccharide vaccine, IV: influenza vaccine, COPD: chronic obstructive pulmonary diseases. Acute exacerbations are classified into two categories: infectious and non-infectious acute exacerbation.

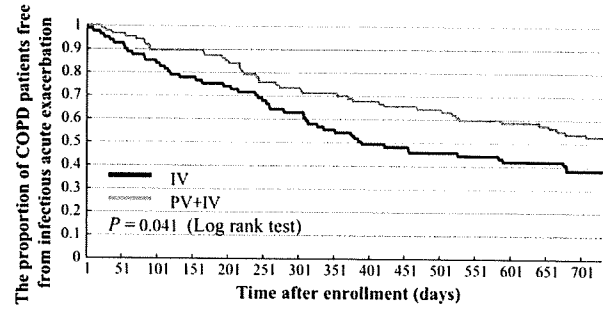

Fig. 3. Kaplan–Meier survival curve for patients with chronic obstructive pulmonary diseases (COPD) showing the proportion of subjects free from infectious acute exacerbation between PV + IV group and IV group during the follow-up period.

The incidence of acute exacerbation (0.53 episodes/(patient year)) in this study was slightly lower than those of previous studies of COPD patients (0.85–1.08 episodes/(patient year)) [27,28]. No significant difference was found in the number of CLD patients with pneumococcal acute exacerbation (P = 0.106) or non-infectious acute exacerbation (P = 0.557) between the two groups (Table 1). In contrast, a significant difference was found in the number of CLD patients with infectious acute exacerbation (P = 0.022) between the two groups (Table 1). The Kaplan–Meier survival curves for CLD patients with infectious acute exacerbation (P = 0.016, Fig. 2B) also demonstrated a significant difference between the two groups. However, no significant difference was found in the mortality during the 2-year period after vaccination in both groups (Table 1).

Furthermore, we examined the number of patients experiencing pneumonia and infectious or non-infectious acute exacerbation in each subcategory (Table 2). No significant difference was found in the number of patients with COPD, SPTB and other CLDs experiencing either pneumonia or non-infectious acute exacerbation. In contrast, a significant difference was found in the number of patients with COPD experiencing infectious acute exacerbation (P = 0.037), but not in the number of patients with SPTB (P = 0.442) or other CLDs (P = 0.599). In COPD patients, the Kaplan–Meier survival curves for infectious acute exacerbation demonstrated a significant difference between the two groups (P = 0.041, Fig. 3), while no significant difference was found in the Kaplan–Meier survival curves for pneumonia (P = 0.543) or non-infectious acute exacerbation (P = 0.426) in COPD patients (data not shown).

While neither a univariate nor multivariate analysis demonstrated a significant association between any variables and the probability of either pneumonia or non-infectious acute exacerbation in either the first or second year, a significant association between the receipt of PV and a low probability of infectious acute exacerbation was found in patients with CLD in the first year, but not in the second year by a single variable analysis (P = 0.019) or multivariate analyses (P = 0.016, Table 3). No significant association was demonstrated between any variable, such as age older than 55 years, age older than 65 years, age older than 75 years or the female sex and the probability for infectious acute exacerbation, in either the first or second year.

The GMCs of serotype-specific IgG for four serotypes in sera from the 35 patients with CLD during the 2 years after their vaccinations are shown in Table 4. Before vaccination, the GMCs ranged from 2.6 μg/ml for serotype 23F to 5.69 μg/ml for serotype 14. One month after vaccination, significant increases in the GMCs of serotype-specific IgG were found for all serotypes in all subjects, compared to those before vaccination (P < 0.01, Table 4). The GMCs of serotype-specific IgG declined below pre-vaccination levels at 6 months post-vaccination for types 6B and 19F. In contrast, the GMCs of serotype-specific IgG remained above the pre-vaccination levels for type 23F and type 14 at 2 years post-vaccination, although they had declined from the immediate post-vaccine levels. Serotype-

Table 3
Estimated result of Cox's proportional hazard model for pneumonia, infectious acute exacerbation, and non-infectious acute exacerbation in patients with chronic lung diseases during the first and the second year

Period	Variables	Pneumonia		Acute exacerbation, infectious		Acute exacerbation, non-infectious	
		Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
First year	PV	0.70 (0.31–1.60)	0.402	0.58 (0.37–0.91)	0.019	1.40 (0.50–3.94)	0.522
	PV	0.72 (0.32–1.68)	0.452	0.56 (0.35–0.90)	0.016	1.56 (0.54–4.52)	0.411
	Age older than 55 years old	N.A.	N.A.	1.02 (0.33–3.12)	0.973	1.97 (0.22–17.4)	0.543
	Age older than 65 years old	1.05 (0.13–8.52)	0.963	1.15 (0.40–3.27)	0.798	0.33 (0.03–3.76)	0.375
	Age older than 75 years old	2.63(0.33–20.8)	0.359	0.92 (0.31–2.75)	0.878	1.97 (0.23–17.1)	0.534
	Female	0.41(0.14–1.23)	0.113	1.01 (0.63–1.61)	0.972	1.01 (0.33–3.08)	0.982
Second year	PV	0.54 (0.13–2.25)	0.397	0.65 (0.26–1.59)	0.342	0.99 (0.28–3.51)	0.987
	PV	0.47 (0.11–2.05)	0.314	0.64 (0.25–1.64)	0.351	1.03 (0.26–3.98)	0.969
	Age older than 55 years old	N.A.	N.A.	1.11 (0.11–10.76)	0.93	1.36 (0.12–14.97)	0.800
	Age older than 65 years old	0.31 (0.31–3.05)	0.315	1.46 (0.18–12.1)	0.725	0.36 (0.03–4.29)	0.418
	Age older than 75 years old	0.60 (0.61–5.77)	0.655	0.81 (0.09–7.73)	0.857	1.09 (0.11–10.78)	0.939
	Female	0.22 (0.27–1.84)	0.163	1.18 (0.47–2.99)	0.72	1.18 (0.32–4.37)	0.797

N.A.: not applicable, PV: pneumococcal polysaccharide vaccine, CI: confidence interval.

Table 4
Serotype-specific IgG in sera from 35 patients with chronic lung diseases before 1 month, 6 months, 1 year and 2 years after vaccination

Serotype	Time point	GMC of IgG (μg/ml) (95% CI)
6B	Pre	3.46(2.51–4.76)
	1 month	5.26(3.78–7.54)*
	6 months	3.51(2.48–5.1)
	1 year	3.41 (2.48–4.85)
	2 year	2.68(1.86–3.97)
14	Pre	5.69(4.44–7.03)
	1 month	12.63(8.76–16.61)*
	6 months	10.35(7.17–13.6)*
	1 year	8.97(6.36–11.47)*
	2 year	7.76(5.53–10.07)*
19F	Pre	4.91 (3.67–6.55)
	1 month	7.05 (5.37–9.3)*
	6 months	4.81 (3.56–6.49)
	1 year	4.60(3.56–5.93)
	2 year	4.58 (3.46–6.05)
23F	Pre	2.60(1.97–3.42)
	1 month	5.82(3.71–9.12)*
	6 months	4.06 (2.72–6.05)*
	1 year	3.56(2.48–5.1)*
	2 year	2.8(1.9–4.1)

GMC: geometric mean concentration, CI: confidence interval.
* $P<0.01$ (for comparison with pre-vaccination value).

specific IgG responses to PV, therefore, were found primarily in sera during the first year, but not during the second year, following vaccination.

4. Discussion

This study demonstrated an additive effect of PV with IV in preventing infectious acute exacerbation, but not pneumonia or non-infectious acute exacerbation, when compared to IV alone in patients with CLD. When theses subjects were divided into three subgroups according to their type of CLD, an additive effect of PV with IV in preventing infectious acute exacerbation was found only in COPD patients, but not in patients with SPTB or other pulmonary diseases. Since no attempt was made to blind the clinical assessors to the vaccine allocation in this study, the possibility of the bias on the clinical assessment obtained by the investigators can not be dismissed.

Although this was an open-label, randomized controlled study on a small scale, the enrolled patients with CLD in this study were at a high risk for pneumonia or acute exacerbation. Since pneumococci has a major role in the development of pneumonia associated with respiratory viruses such as influenza virus, these viruses contribute to the pathogenesis of bacterial pneumonia among children [29]. Other investigators have also demonstrated that influenza neuraminidase facilitated bacterial adherence of *S. pneumoniae*, and resulted in secondary bacterial pneumonia in mice [30,31]. Based on the interaction between influenza virus and pneumococci on the pathogenesis of bacterial pneumonia, an additive or synergistic effect of the combined vaccination with PV and IV in preventing pneumococcal pneumonia can be expected.

A large prospective study in Sweden reported the additive effects of PV with IV in reducing pneumococcal pneumonia as well as invasive pneumococcal diseases, compared to no vaccination, among adults aged 65 years or older during the first 6 months after vaccination [32]. The authors demonstrated the additive effect of PV with IV only in reducing the hospital mortality due to pneumonia, compared to a vaccination with IV alone or PV alone, at 1-year after vaccination [20]. Another investigator, however, did not find an additive effect of PV with IV in preventing pneumococcal pneumonia as well as pneumonia, compared to IV alone, among the same population during a 2-year period after vaccination [33].

A double-blind, randomized placebo-controlled trial in Thailand recently reported that IV was found highly effective in preventing the influenza-related acute respiratory illness, but not acute exacerbation or pneumonia, in COPD patients [23]. Since no effect of IV was found for preventing acute exacerbation among patients with COPD, our data suggests not only the importance of PV in addition to IV among patients with CLD, but also the role of pneumococcal infection in the pathogenesis of acute exacerbation in such patients.

Although no significant difference was found in the number of patients with pneumococcal acute exacerbation between the two groups in this study, a reduced number of pneumococcal infections in the PV + IV group may suggest the contribution of protective immunity raised by PV in such patients. More importantly, the additive effect of PV with IV on infectious acute exacerbation was significant during the first year, but not the second year after vaccination. This effect was associated with an immune response of serotype-specific IgG to PV, which was prominent in all serotypes during the first year.

Although the protective concentrations of serotype-specific IgG are not known, most of CLD patients showed a level of IgG much higher than the threshold (0.35 μg/ml) that predicts protection in infants against invasive disease at a population level after immunization with pneumococcal conjugate vaccine in this study [34]. A recent study also reported that higher levels of antipneumococcal antibodies did not correlate with protection from pneumococcal colonization in patients with COPD [35]. Therefore, the question arises as to why an additional increase of IgG in sera is required for preventing infectious acute exacerbation in COPD patients. A recent report demonstrated that the levels of anti-serotype-1 IgG in bronchoalveolar lavage (BAL) fluid were less than 10 ng/ml in 25 of 49 HIV-uninfected adults, while the mean level of serotype-1 specific IgG in sera of such subjects was 1608 ng/ml [36]. Thus, the level of specific IgG in the neat BAL fluid is at least 161 times lower than that in sera. According to this information and the data of levels of specific IgG in sera of patients with CLD before (2.60–5.69 μg/ml) and after vaccination (5.26–12.63 μg/ml) in this study, the levels of specific IgG in BAL fluid are estimated to be less than 35.3 ng/ml before vaccination and less than 78.5 ng/ml after vaccination, respectively. Since we found a significant effect of PV + IV on infectious acute exacerbation, compared with IV alone, the levels of serotype-specific IgG that rise higher than approximately 35 ng/ml in the lower airway fluid may be critical for preventing infectious acute exacerbation in patients with COPD.

Furthermore, the cost of acute exacerbation on patients with COPD creates a significant economic impact [3]. The literatures reports that the estimated costs for hospitalization due to acute exacerbation will range from $5655 to $7413 in developed countries [37–39]. The reduced frequency (27.2%) of acute exacerbation in the PV + IV group in this study, compared to the IV group, has significant economic implications for patients with COPD.

In conclusion, this pilot study demonstrated an additive effect of PV in combination with IV on infectious acute exacerbation of patients with COPD. This effect was closely associated with serotype-specific immune response to PV that was primarily found during the first year after vaccination. Further studies on a larger scale are required to investigate the additive effects of PV and IV on the incidence of acute exacerbation in COPD patients with various levels of airflow limitations.

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Induction of opsonophagocytic killing activity with pneumococcal conjugate vaccine in human immunodeficiency virus-infected Ugandan adults

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ABSTRACT

The levels of IgG determined by ELISA may have limited relevance in human immunodeficiency virus (HIV)-infected adults because of non-functional antibodies. 58 HIV-1-infected and 29 HIV-uninfected Ugandan adults were immunized with conjugate vaccine (CV) followed by polysaccharide vaccine (PV) after a 2-month interval, and the opsonophagocytic killing (OPK) titers against serotype 4 or 14 pneumococcal strains as well as the levels of serotype-specific IgG in sera were determined. Significant increases were found in the OPK titers and IgG levels for both serotypes after CV vaccination irrespective of HIV status. Increases in IgG levels and OPK titers were largely dependent on the $CD4^+$ cell counts, except for increases in the IgG levels for serotype 4. The proportions with serum OPK titer equal to or greater than 8 were 0–4.3% for serotype 4 and 26.7–42.9% for serotype 14 before vaccination, but the proportions increased up to 43.3–86.2% for serotype 4 and 63.3–96.6% for serotype 14 in all three groups 2 months after CV vaccination. The serum OPK titers remained at levels higher than the pre-vaccination level for at least 8 months after CV vaccination. A single dose of CV could afford some protective immunity in HIV-infected African adults before the introduction of antiretroviral therapy.

1. Introduction

More than 60% of the 40 million people living with HIV in the world are in sub-Saharan Africa [1]. HIV infection is, therefore, a major health concern facing adults in this area. The authors' previous study of community-acquired pneumonia (CAP) among adults in Uganda revealed a high prevalence of HIV-1 infection among these patients [2]. The majority of HIV-1-infected patients with CAP have peripheral CD4 counts below 400 μl^{-1}. *Streptococcus pneumoniae* (*S. pneumoniae*) was found to be a common pathogen, which was isolated in approximately 40% of bacterial CAP. The host defense against *S. pneumoniae* depends largely on opsonophagocytic killing antibodies to capsular polysaccharide (CPS) [3,4], although an antibody-independent, $CD4^+$ T cell-dependent mechanism of protection has been reported in mouse model [5,6]. Low opsonic activity in the sera of HIV-infected African adults, therefore, predisposes them to a serious risk of invasive pneumococcal infections [7–9].

Approaches to prevent bacterial pneumonia in adults with HIV infection involve antimicrobial prophylaxis, highly active antiretroviral therapy (ART) and vaccination [10]. Although a significant decrease in the rates of bacterial pneumonia in HIV-infected adults after antimicrobial prophylaxis with co-trimoxazole has been reported in Cote, d'Ivoire [11], further studies are required to confirm its effects in preventing bacterial pneumonia even in areas where penicillin-resistant pneumococci are highly prevalent in the population [12]. ART can prevent bacterial pneumonia in HIV-infected adults in developed countries, but this has not been confirmed in developing countries [13]. Although the WHO recommends that HIV-infected adults and adolescents, who are either in WHO clinical staging 4 or have a CD4 cell count below 200 μl^{-1} start

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ART [1], an access to ART is limited in sub-Saharan Africa including Uganda [14]. The WHO estimates that only 20% of the estimated 6.5 million people in low and middle-income countries who are in urgent need of ART were receiving ART at the end of 2005 [15].

Previous studies reported the effects of pneumococcal polysaccharide vaccine (PV) in HIV-infected African adults [16,17]. A clinical study of PV in HIV-infected Ugandans, however, has demonstrated an increased risk of invasive pneumococcal infection and no protection against all-cause pneumonia [18], and the same group subsequently reported a 16% reduction in all-cause mortality after PV vaccination [19]. The use of PV in HIV-infected African adults is therefore inconclusive. On the other hand, previous studies in the Netherlands, US and Uganda reported promising effects of pneumococcal conjugate vaccine (CV), which induced high levels of serotype-specific IgG among HIV-infected adults [20–22]. Furthermore, an enhanced antibody response to certain serotypes has been shown in HIV-infected adults following immunization with PV after prior immunization with CV [20,23]. The concentration of serotype-specific IgG determined by ELISA, however, may have limited relevance in HIV-infected adults and children because of non-functional anti-CPS IgG [6,7,24,25]. Analysis of serum opsonophagocytic killing (OPK) activity is, therefore, required to study the immunogenicity of CV in HIV-infected adults. This study was designed to determine whether immunization with CV followed by PV could increase the levels of serotype-specific IgG and OPK titers against *S. pneumoniae* in sera of HIV-infected African adults before the introduction of ART.

2. Materials and methods

2.1. Subjects

After providing written informed consent, HIV-1-infected and HIV-uninfected asymptomatic adults were enrolled by two physicians at the Joint Clinical Research Centre (JCRC), Kampala between February and June 2005. HIV-1 infection was determined by a screening EIA (HIV1&2 Rapid Serotest, Healthcare Technologies Ltd., Ashdod, Israel) and a second EIA for confirmation (Vironostika HIV Uni-FormII Ag/Ab, bioMétrieux, Marcy l'Etoile, France). Study subjects were classified into three subgroups: group I (HIV-1-infected adults with peripheral $CD4^+$ cell count: 200–499 μl^{-1}; $n=30$), group II (HIV-1-infected adults with peripheral $CD4^+$ cell count: $\geqq 500\,\mu l^{-1}$; $n=28$) and group III (HIV-uninfected adults; $n=29$) (Table 1). Plasma HIV-1 RNA loads were quantified as described elsewhere for the total of 58 HIV-infected adults [26]. Enrolling 90 subjects in three study groups would have given the study 70% power at an alpha level of 0.05 to detect a significant increase in the percentage of persons responding with a $\geqq$2-fold rise in the serum levels of serotype-specific IgG from 42% to 74% between groups of HIV-infected and HIV-uninfected adults [27].

2.2. Immunization of study subjects with pneumococcal vaccines

Seven-valent CV (Prevenar®, Wyeth-Lederle), containing 2 μg of six serotypes (4, 9V, 14, 18C, 19F and 23F) and 4 μg of CPS from 6B covalently linked to a CRM_{197}, non-toxic mutant diphtheria toxin, was given as an intradeltoid injection to all of the study subjects. Two months after CV vaccination, 23-valent PV (Pneumovax, Merck-Banyu), which contains 25 μg of each of 23 serotypes was given as an intradeltoid injection to all of the study subjects. All of the study subjects were requested to visit the outpatient department of JCRC, and be examined by two physicians immediately before and 2, 3 and 8 months after CV vaccination. Serum samples were also obtained at the time of the scheduled visits throughout the study, and stored at −80 °C until use. When they had new symptoms, the participants were asked to visit the outpatient department of the JCRC for medical examination by the physicians. At 8 months post-CV vaccination, the peripheral $CD4^+$ cell count was reexamined in HIV-infected adults. While all of HIV-infected subjects were successfully followed for the study period, six of the HIV-uninfected healthy control subjects were lost by the time of follow-up. Sera were subsequently collected from 23 HIV-uninfected subjects 8 months after CV vaccination.

2.3. Determination of serotype-specific IgG levels

Since preabsorption of serum with both cell wall polysaccharide (PS) and 22F PS is necessary to remove non-specific antibodies in HIV-infected adults and HIV-uninfected adults, the concentrations of serotype-specific IgG were measured using the US reference pneumococcal antiserum (89 SF-3), courtesy of Dr. Carl Frasch, as previously described [28,29]. Among 29 pneumococcal isolates known as etiologic agents of CAP in Ugandan adults [2], 5 strains of serotype 4 and 3 strains of serotype 14 were identified (unpublished data). Therefore, we decided to use ELISA to determine the IgG levels in sera for serotypes 4 and 14 CPS, which are major serotypes in Uganda.

2.4. Differentiation of HL-60 cells and OPK assay

Undifferentiated HL-60 cells (Cell number; JCRB0085, Health Science Research Resources Bank, Japan) were cultured in RPMI-1640 medium with 10% fetal calf serum (FCS) (Hyclone, South Logan, UT). HL-60 cells were differentiated into granulocytes in the presence of 1 μM all-*trans*-retinoic acid (Sigma Chemical Co., St. Louis, MO) in RPMI-1640 medium with 20% FCS for 3 days [30,31]. Cells were washed twice by centrifugation, and the supernatant was removed. 20 μl of PE-conjugated mouse anti-human CD11b monoclonal IgG_1 (BD PharMingen, San Diego, CA) or PE-conjugated mouse monoclonal IgG_1 isotype control (Dakocytomation AS, Glostrup, Denmark) were added to each tube and incubated at 4 °C for 30 min. Samples were washed twice and resuspended in PBS containing 1% paraformaldehyde, and analyzed by flowcytometry

Table 1
Clinical characteristics and laboratory data for 58 HIV-1-infected and 29 HIV-uninfected adults

Characteristic	Time point	Group I ($n=30$) HIV-1$^+$, CD4: 200–499 μl^{-1}	Group II ($n=28$) HIV-1$^+$, CD4: $\geqq 500\,\mu l^{-1}$	Group III ($n=29$) HIV-1$^-$
Age, mean years ± S.D.	Pre	36.77 ± 7.45[a]	37.11 ± 7.63[a]	26.83 ± 7.70
Peripheral $CD4^+$ cell count, mean cell/μl ± S.D.	Pre	352.00 ± 80.71[a,b]	720.25 ± 170.69[c]	882.45 ± 257.45
log_{10} plasma HIV RNA load copies/ml ± S.D.	Pre	4.81 ± 0.85[b]	3.84 ± 0.83	ND
Peripheral $CD4^+$ cell count, mean cell/μl ± S.D.	8 m post-CV	362.41 ± 155.82[b]	707.39 ± 213.79	ND

ND: Not done.
[a] $P<0.001$ (vs. group III).
[b] $P<0.001$ (vs. group II).
[c] $P<0.01$ (vs. group III).

(FACSCalibur, BD Biosciences, San Jose, CA). While no expression of CD11b was found in the undifferentiated cells, increased expression of CD11b was found in the differentiated cells by retinoic acid (data not shown). The expression of CD11b was specific because no increase was found in the fluorescent intensity of the differentiated cells stained with PE-conjugated control antibody.

OPK titer against *S. pneumoniae* was measured as described elsewhere with some modification [32]. Briefly, the differentiated HL-60 cells were used at an effector/target cell ratio of 400/1. 10 μl of serially diluted serum sample was aliquoted into each well of 96-well microtiter plate. *S. pneumoniae* serotype 4 (strain P-03-106) or serotype 14 (strain P-03-170), both isolated from patients with CAP, were used for this assay. In addition, we also employed two reference strains (DS2382-94 for serotype 4 and DS2214-94 for serotype 14) as standard quality control strains for this assay [32]. These reference strains were gifts from Dr. Moon H. Nahm, University of Alabama at Birmingham. 20 μl of bacterial suspension (~10^3 cfu) was added to each well. Then the plate was allowed to incubate at 37 °C in a 5% CO_2 atmosphere for 15 min. Following this incubation period, 10 μl of rabbit complement (Dynal Biotech Inc., Lake Success, NY) was added to each well. Then, 40 μl of washed cells (4×10^5 cells) was immediately added to each well. The assay plate was incubated at 37 °C for 45 min with horizontal shaking (220 rpm). A 5 μl aliquot from each well was plated onto an agar medium plate. Plates were incubated overnight and viable colonies were counted for each well. Typically, 10–60 colonies were counted. The OPK titers were expressed as the reciprocal of the serum dilution with ≥50% killing compared with the growth in the complement control wells. Serum samples with titers of <8 were reported as a titer of 4 for analysis of the levels of serotype-specific IgG, but excluded for analysis of the correlation between the levels of IgG or HIV-1 viral load and the serum OPK titers. Quality control sera (sera with a known titer) were added to each plate and the blinded test samples for OPK titer were examined only when the titers of quality control sera were identical. Functional activity of serotype-specific IgG was expressed as a concentration of IgG required for 50% killing of pneumococcal strain by dividing the IgG concentration of a test sample by OPK titer.

2.5. Statistical analysis

The subjects' ages, $CD4^+$ cell count and levels of HIV-1 viral load were compared by one-way analysis of variance and by multiple comparison methods. Differences in geometric mean concentrations (GMCs) of serotype-specific IgG, OPK titers and the ratios of OPK titer to serotype-specific IgG were assessed using the Friedman and Steel-Dwass tests [33]. The significance of the correlation was estimated using Spearman's rank correlation. Data were considered significant when $P < 0.05$.

3. Results

No difference was found in age between groups I and II, but differences in age were significant between either of group I or II and the group III ($P < 0.001$, Table 1). Differences in $CD4^+$ cell count and the level of HIV-1 viral load were significant between groups I and II ($P < 0.001$), which is in agreement with our previous report [26]. No significant decrease in the $CD4^+$ cell count was found in either group I or II during the 8 months post-CV vaccination. ART was initiated in one case (32 years old, male) in the group I at 7 months post-CV vaccination because of recurrent pneumonia. The $CD4^+$ cell count and plasma $\log_{10}$ HIV viral load of this case were 212 μl^{-1} and 5.78, respectively, at the time of enrollment.

No significant difference was found in the GMCs of IgG specific to either serotype 4 or 14 in sera among the three groups before vaccination (Table 2). Two months after CV vaccination, significant increases in the GMCs of IgG specific to both serotypes 4 and 14 were found for all three groups, compared to those before vaccination ($P < 0.01$).

No significant difference was found in the GMCs of IgG specific to serotype 4 among the three groups 2 months after CV vaccination ($P = 0.23$). In contrast, the GMCs of IgG specific for serotype 14 were highest in group III, followed by group II, with group I having the lowest GMC 2 months after CV vaccination, and the difference between groups I and III was significant at this time point ($P < 0.05$). One month post-vaccination with PV (3 months after CV vaccination), however, no significant increase was found in the GMCs of IgG specific to either serotype 4 or 14 compared to those at 2 months after vaccination with CV in each group. Although the GMCs of IgG specific to serotype 14 largely declined at 8 months post-vaccination with CV, no significant decrease in the GMCs of IgG specific to serotypes 4 was found in any group between 2 months after CV vaccination and 8 months after CV vaccination. The GMCs of IgG specific to both serotypes 4 and 14 were still higher 8 months after CV vaccination than before vaccination. In addition, no correlation was found between the fold increase of specific IgG to either serotype 4 or 14 after CV vaccination and the levels of HIV-1 viral load in HIV-infected adults.

The concentration of 0.35 μg/ml for serotype-specific IgG has been suggested by world health organization (WHO) working group as a putative measure of protection against invasive disease in infants at a population level after immunization with pneumococcal conjugate vaccine [34]. This working group reported that antibody concentrations of 0.2–0.35 μg/ml correlated best with an

Table 2
Comparison of geometric mean concentrations of serotype-specific IgG in sera from HIV-1-infected patients and HIV-uninfected adults before vaccination and after vaccination

Serotype	Time point	GMC of IgG (μg/ml) (95% CI) in sera from		
		Group I ($n = 30$) HIV-1^+, CD4: 200–499 μl^{-1}	Group II ($n = 28$) HIV-1^+, CD4: ≧500 μl^{-1}	Group III ($n = 29$) HIV-1^-,
4	Pre	1.55 (1.15–2.08)	0.82 (0.58–1.18)	0.83 (0.59–1.16)
	2 m post-CV	4.17 (3.03–5.73)[a]	4(2.78–5.74)[a]	6.75 (4.78–9.52)[a]
	3 m post-CV (1 m post-PV)	4.56 (3.51–5.92)[a]	3.88 (2.73–5.52)[a]	6.87 (4.89–9.66)[a]
	8 m post-CV	5.52 (4.41–6.9)[a]	4.07 (3.19–5.19)[a]	4.64 (3.60–5.98)[a,d]
14	Pre	5.27 (3.76–7.37)	5.1 (3.40–7.66)	4.67 (2.75–7.94)
	2 m post-CV	25.31 (16.79–38.14)[a,b]	42.44 (23.41–76.95)[a]	52.11 (31.41–86.45)[a]
	3 m post-CV (1 m post-PV)	23.14 (15.96–33.55)[a,c]	42.76 (23.98–76.24)[a]	53.86 (34.11–85.05)[a]
	8 m post-CV	17.73 (12.68–24.79)[a,c]	28.66 (17.18–47.81)[a]	37.8 (24.99–57.18)[a,d]

CI: Confidence interval; Pre: pre-vaccination; CV: pneumococcal conjugate vaccine; PV: pneumococcal polysaccharide vaccine.
[a] $P < 0.01$ (vs. pre-vaccination).
[b] $P < 0.05$ (vs. group III).
[c] $P < 0.01$ (vs. group III).
[d] $n = 23$ in group III.

OPK titer of 8 [34], which in turn correlated best with protective efficacy in infants. The proportions of subjects with IgG specific to serotype 4 of ≧0.35 μg/ml were 100% in group I, 85.7% in group II and 79.3% in group III before vaccination and 100% for all groups 2 months after CV vaccination in this study. The proportions of subjects with IgG specific to serotype 14 of ≧0.35 μg/ml were 100% in groups I and II and 96.4% in the group III, before vaccination and 100% for all groups 2 months after CV vaccination.

The geometric means of serum OPK titers for the serotypes 4 and 14 were similarly low in sera of all three groups before vaccination (Table 3). The differences among the three groups were not statistically significant. Two months after CV vaccination, significant increases in the serum OPK titers for both serotype strains 4 and 14 compared to pre-vaccination levels were found in all three groups ($P<0.01$). The serum OPK titers for both serotypes 4 and 14 were highest in group III followed by group II, with group I having the lowest titers 2 months after CV vaccination. Serum OPK titers for both serotypes 4 and 14 differed significantly between groups I and III at 2, 3 and 8 months after vaccination with CV ($P<0.01$). Group II had significantly higher OPK titers than group I for serotype 4 at 3 and 8 months post-vaccination with CV, and for serotype 14 at 8 months post-vaccination with CV ($P<0.05$). In additional experiments, the OPK titers of nine serum samples (three from each group) were determined using strains of DS2382-94 and DS2214-94 which are widely used as target strains for serotypes 4 and 14, respectively. The values of serum OPK titer were identical or close to those determined using the non-reference strains (data not shown).

The proportions of subjects with a serum OPK titer of ≧8 were very low (0–4.3%) for serotype 4 in all three groups before vaccination (Fig. 1). On the other hand, the proportion was lower in group I (26.7%) than those in group II (42.9%) or group III (41.4%) for serotype 14 before vaccination. In contrast, marked increases in the proportion with a serum OPK titer of ≧8 were found for both serotypes in all three groups two months after CV vaccination. The proportion of the serum OPK titer of ≧ 8 was highest in group III, followed by group II and group I for both serotypes at 2 and 3 months post-CV vaccination. Eight months after CV vaccination, the proportions of subjects with a serum OPK titer of ≧8 decreased in groups I and II for both serotypes.

Since few serum samples contained detectable OPK titers for serotype 4 before vaccination (one in group I, none in group II and three in group III), correlations were not assessed between the levels of serotype-specific IgG and the serum OPK titers. On the other hand, the concentrations of serotype-specific IgG significantly correlated with OPK titers for serotype 14 before vaccination in groups II ($r=0.61$, $P<0.05$, $n=14$) and III ($r=0.61$, $P<0.05$), but not in group I ($r=0.56$, $P=0.08$, $n=11$). Two months after CV vaccination, significant correlations between two parameters were found for serotype 4 in groups I ($r=0.74$, $P<0.01$, $n=15$) and III ($r=0.67$, $P<0.001$, $n=26$), but not in group II ($r=0.27$, $P=0.2$, $n=23$) and for serotype 14 in groups II ($r=0.85$, $P<0.001$, $n=23$) and III ($r=0.38$, $P=0.05$, $n=28$), but not in group I ($r=0.44$, $P=0.06$, $n=11$).

The GMCs of serotype-specific IgG required for 50% killing ranged 136–601 ng/ml in the group I, 79–377 ng/ml in the group II and 72–316 ng/ml in the group III, although few data are available for serotype 4-specific IgG before vaccination (Table 4). No

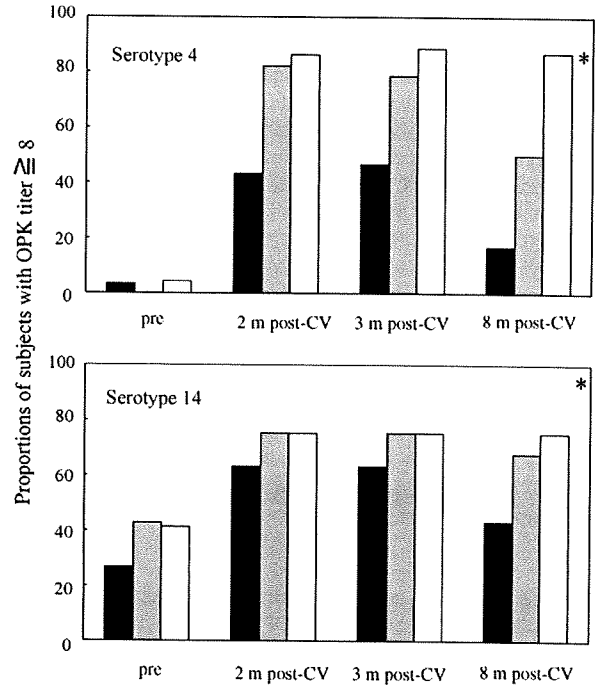

Fig. 1. The proportion of subjects with opsonophagocytic killing (OPK) titers equal to or greater than 8 for serotype 4 (upper panel) and serotype 14 (lower panel) in groups I ($n=30$, closed bar), II ($n=28$, grey bar), and III ($n=29$, open bar) before and 2, 3 and 8 months after vaccination with pneumococcal conjugate vaccine (CV). *$n=23$ in group III at 8 months after CV vaccination.

Table 3
Comparison of geometric mean opsonophagocytic killing (OPK) titers of sera from HIV-1-infected and HIV-uninfected adults before vaccination and after vaccination

Serotype	Time point	GM OPK titer (95% CI) of sera from		
		Group I ($n=30$) HIV-1$^+$, CD4: 200–499 μl^{-1}	Group II ($n=28$) HIV-1$^+$, CD4: ≧500 μl^{-1}	Group III ($n=29$) HIV-1$^-$
4	Pre	4.29 (3.72–4.94)	4 (4–4)	4.62 (3.76–5.66)
	2 m post-CV	13.93 (7.78–24.94)[a,b]	32 (19.08–53.68)[a,c]	75.66 (45.15–126.77)[a]
	3 m post-CV (1 m post-PV)	13.61 (7.91–23.41)[a,b,e]	37.12 (22.21–62.07)[a]	73.87 (45.71–119.36)[a]
	8 m post-CV	6.81 (4.61–10.04)[b,d,e]	18.11 (10.18–32.22)[a]	44.58 (26.55–74.84)[a,g]
14	Pre	7.29 (5.10–10.43)	12.8 (7.52–21.82)	12.01 (6.94–20.78)
	2 m post-CV	41.26 (19.57–87.01)[a,c]	107.63 (51.49–225.02)[a]	275.03 (173.88–435.03)[a]
	3 m post-CV (1 m post-PV)	43.21 (20.42–91.46)[a,b]	118.84 (57.32–246.38)[a]	281.68 (181.69–436.72)[a]
	8 m post-CV	13.61 (7.62–24.3)[b,e]	47.55 (22.52–100.42)[d]	116.94 (69.96–195.45)[a,f,g]

CI: Confidence interval; Pre: pre-vaccination; CV: pneumococcal conjugate vaccine; PV: pneumococcal polysaccharide vaccine.
[a] $P<0.01$ (vs. pre-vaccination).
[b] $P<0.01$ (vs. group III).
[c] $P<0.05$ (vs. group III).
[d] $P<0.05$ (vs. pre-vaccination).
[e] $P<0.05$ (vs. group II).
[f] $P<0.05$ (vs. 2 and 3 months post-CV).
[g] $n=23$ in group III.

Table 4
Comparison of geometric mean concentration (GMC) of serotype-specific IgG required for 50% killing (OPK) titers of sera from HIV-1-infected and HIV-uninfected adults before vaccination and after vaccination

Serotype	Time point	GMC of IgG (ng/ml) required for 50% killing (95% CI) [no. of subjects evaluated] in sera from		
		Group I ($n=30$) HIV-1$^+$, CD4: 200–499 μl^{-1}	Group II ($n=28$) HIV-1$^+$, CD4: ≧500 μl^{-1}	Group III ($n=29$) HIV-1
4	Pre	284 (NA) [1]	NA (NA)	179 (9–3492) [3]
	2 m post-CV	136 (80–234) [14]	102 (68–155) [23]	72 (52–100) [26]
	3 m post-CV (1 m post-PV)	137 (80–234) [15]	79 (56–111) [23]	76 (55–106) [26]
	8 m post-CV	201 (57–706) [7]	86 (46–161) [16]	96 (56–163) [22][a]
14	Pre	441 (236–825) [11]	248 (139–441) [14]	307 (149–631) [15]
	2 m post-CV	302 (182–501) [19]	279 (204–382) [23]	176 (101–305) [28]
	3 m post-CV (1 m post-PV)	229 (142–372) [19]	246 (163–369) [23]	174 (106–288) [28]
	8 m post-CV	601 (364–992) [14][b]	377 (269–529) [18]	316 (207–481) [22][a]

CI: Confidence interval; Pre: pre-vaccination; CV: pneumococcal conjugate vaccine; PV: pneumococcal polysaccharide vaccine; NA: not available.
[a] $n=23$ in group III.
[b] $P<0.05$ (vs. 3 m post-CV).

significant difference was found in the GMCs of serotype-specific IgG required for 50% killing in sera among the three groups before and after vaccination. No significant difference was also found in the GMCs of serotype-specific IgG required for 50% killing of each group among different time points, with a exception of a significant difference in those of group I between 3 months after CV vaccination and 8 months after CV vaccination ($P<0.05$). No significant effect of vaccination with PV following CV was found in the serum OPK titers for either serotype 4 or 14 in any group. Eight months after CV vaccination, the serum OPK titers for serotypes 4 and 14 largely declined compared to those 2 months after CV vaccination. The OPK titers for serotypes 4 and 14 in sera of the three groups, however, remained higher than pre-vaccination levels at 8 months post-vaccination with CV. In addition, no correlation was found between the increase in serum OPK titers for both serotypes 4 and 14 after CV vaccination and the levels of HIV-1 viral load in HIV-infected adults.

4. Discussion

In this study, it has been demonstrated that a single dose of CV can increase serum OPK titers against pneumococcal strains as well as the levels of serotype-specific IgG, in HIV-uninfected and HIV-infected Ugandan adults with peripheral CD4$^+$ cell counts of ≧200 μl^{-1} for at least 8 months after CV vaccination. HIV-infected adults with peripheral CD4$^+$ cell counts less than 200 μl^{-1} who were at risk for pneumococcal infections were not involved in this study, because they are recommended to receive ART [1] and are known to be poorly responsive to CV [20,27]. Serotype 14-specific IgG response to a single dose of CV was dependent on CD 4$^+$ cell counts in HIV-infected adults, which was in agreement with previous reports [20,22], but the magnitude of IgG response to CV in our study was higher than that shown in a previous study [22]. In contrast, serotype 4-specific IgG response to a single dose of CV was independent of CD4$^+$ cell counts in our study: this finding was also consistent with a previous study [22].

The major limitation in this study may be the lack of an unvaccinated control group. Pneumococcal pneumonia or nasopharyngeal colonization may increase the levels of serotype-specific IgG in sera from subjects, in addition to the immunological effects by vaccination, during the study period [35]. Since only five episodes of pneumonia with unknown etiology were identified in the HIV-infected and HIV-uninfected groups (two episodes in group I, one episode in group II and two episodes in group III) during the 8 months (data not shown), we speculate that the possibility of active immunization by pneumococcal pneumonia among study participants was low in this study. Since the nasopharyngeal carriage of pneumococcal strains was not examined in this study, the influence of nasopharyngeal carriage on the levels of IgG and OPK titers in serum cannot be dismissed.

Although a previous study reported an immunological effect of a CV primed PV booster with a 4-week interval in HIV-infected adults [23], no effects of PV following CV vaccination were found in the levels of serotype 4 or 14-specific IgG in our study. The lack of additional immune response after PV following CV could be explained by the differences in races and ages of the participants and in the timing of the PV boost 2 months following CV in this study [23]. Another reason for the lack of additional immune response by PV might be the increased levels of serotype 4 or 14 specific IgG achieved by CV, because a recent study similarly demonstrated that elderly subjects with a serotype-specific IgG higher than 5 μg/ml in sera before vaccination tended to respond to PV at a lower magnitude [36]. The undiminished persistence of the levels of OPK titers and of IgG shown in this study up to 8 months after CV vaccination may, in part, be explained by the boosting effect of nasopharyngeal carriage of pneumococcal strains [37].

The general consensus on measuring the serum OPK titers encouraged the authors to develop this assay for the evaluation of immunogenicity in this study [24]. Although an OPK assay for the measurement of functional antibody activity has been available [32], there were difficulties in achieving differentiation of HL-60 cells into granulocytes in the presence of dimethylformamide as a previous study indicated [38]. In this study, it has been shown that differentiated HL-60 cells by the use of retinoic acid can be applicable for an OPK assay against *S. pneumoniae* for evaluation of the immunogenicity of CV.

While the proportions of subjects with serotype-specific IgG of ≧0.35 μg/ml were higher than 80% and 95% for serotypes 4 and 14 among HIV-infected and HIV-uninfected Ugandan adults in this study, a previous study demonstrated that the proportions of subjects with serotype-specific IgG were less than 10% for serotype 4 and less than 30% for serotype 14 among unvaccinated HIV-infected African children [25]. A relatively low proportion of subjects, however, exhibited OPK titers of ≧8 for serotypes 4 and 14 in both HIV-infected and -uninfected Ugandan adults before vaccination in this study. Although WHO working group reported that 0.2–0.35 μg/ml for the levels of serotype-specific IgG correlate with an OPK titer of 8 in infants [34], this threshold may not be applicable for unvaccinated adults.

In this study, we determined the levels of serotype-specific IgG, but not IgM or IgA. While a poor correlation was found between the opsonic activity and the levels of either CPS-specific IgM or IgA, the serum opsonic activity correlated best with the levels of CPS-specific IgG in healthy adults following CV vaccination [39,40]. The levels of IgG significantly correlated with the OPK titers for serotype 4, except for group II, and for serotype 14, except for group I, 2

months after CV vaccination in this study. Furthermore, the GMCs of serotype-specific IgG required for 50% killing ranged 79–601 ng/ml in HIV-infected adults and 72–315 ng/ml in HIV-uninfected adults after a single dose of CV vaccination in this study. In contrast, the GMCs of serotype-specific IgG required for 50% killing ranged 2–119 ng/ml in infant populations after CV vaccination [41–43]. Collectively, our present data suggest that opsonic function of serotype-specific IgG after CV vaccination is much decreased in Ugandan adults, irrespective of HIV infection, than those in infants.

In conclusion, this study document the immunogenicity of a single dose of CV on the OPK titer and the levels of serotype-specific IgG in sera of HIV-infected Ugandan adults with $CD4^+$ cell count higher than $200\,\mu l^{-1}$. A single dose of CV increased the serum OPK activity as well as the levels of IgG, and maintained them above their pre-vaccination levels for at least 8 months after CV vaccination. A single dose of CV could increase protective immunity in HIV-infected African adults with $CD4^+$ cell count higher than $200\,\mu l^{-1}$ before the introduction of ART. A clinical trial of the efficacy of CV in this population is needed in sub-Saharan Africa.

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