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Original Article

Streptococcus pneumoniae colonization among patients with human immunodeficiency virus–1 who had received 23-valent polysaccharide pneumococcal vaccine

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Background and purpose: To evaluate the impact of 23-valent polysaccharide pneumococcal vaccine on *Streptococcus pneumoniae* colonization in the upper airway of patients with human immunodeficiency virus (HIV)–1 in Taiwan.

Methods: 302 HIV-infected patients aged 15 years or older underwent swab cultures of the posterior pharynx and tonsils for *S. pneumoniae* between June 1, 2000 and June 30, 2002. 198 patients (65.6%) had received 23-valent polysaccharide pneumococcal vaccine with a median interval of 420 days (range, 27-634 days) before cultures were performed. Clinical characteristics and laboratory findings were analyzed to determine the factors associated with *S. pneumoniae* colonization in the upper airway.

Results: Twenty patients (6.6%) had colonization with *S. pneumoniae*: 15 of 198 patients (7.6%) who had received 23-valent polysaccharide pneumococcal vaccine and 5 of 104 patients (4.8%) who had not received the vaccine (p = 0.37). According to the multivariate analysis, smoking was the only factor that was statistically significantly associated with *S. pneumoniae* colonization (adjusted odds ratio, 4.03; 95% confidence interval, 1.04-15.64; p = 0.04); pneumococcal vaccination was not statistically significantly associated with *S. pneumoniae* colonization.

Conclusions: Among HIV-infected patients, smoking was the only factor significantly associated with a higher prevalence of upper airway colonization by *S. pneumoniae*. As previous receipt of 23-valent polysaccharide pneumococcal vaccine was not associated with a lower prevalence of *S. pneumoniae* colonization, a better vaccination strategy, in addition to smoking cessation, may be needed to reduce *S. pneumoniae* colonization in HIV-infected adults.

Key words: Acquired immunodeficiency syndrome; Antiretroviral therapy, highly active; HIV infections; HIV-1; Pneumococcal vaccines; *Streptococcus pneumoniae*

Introduction

Patients with human immunodeficiency virus (HIV) infection are at higher risk for invasive infections due to *Streptococcus pneumoniae* than people without HIV infection. Rates of invasive pneumococcal infections among HIV-infected patients may be as high as 46-fold greater than among people without HIV [1]. Although observational and case-control studies have shown that pneumococcal vaccination and highly active antiretroviral therapy (HAART) are associated with decreased risk for invasive pneumococcal infections among HIV-infected patients [2-5], whether pneumococcal vaccination decreases *S. pneumoniae* colonization among HIV-infected adults receiving HAART remains unclear. Nearly all of the studies investigating pneumococcal

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colonization among HIV-infected patients were conducted among children who had not received vaccination or HAART [6-9], or in adults who had received pneumococcal vaccination but not HAART [10,11]. The only study conducted in HIV-infected adults receiving HAART did not show a reduced risk for *S. pneumoniae* colonization in patients who had received pneumococcal vaccination. However, only 27% of the participants had received pneumococcal vaccination [12]. In this cross-sectional survey, the association between receipt of 23-valent polysaccharide pneumococcal vaccine and *S. pneumoniae* colonization in the upper airway was evaluated among HIV-infected patients treated at the National Taiwan University Hospital, Taipei, Taiwan, the largest referral hospital for HIV inpatient and outpatient care in Taiwan.

Methods

Study population

308 consecutive non-hemophiliac HIV-infected patients aged 15 years or older who received medical care at the National Taiwan University Hospital underwent surveillance swab cultures of the posterior pharynx and tonsils using a dry sponge swab (EZ Culturette; Becton Dickinson, Sparks, MD, USA). Between June 1, 2000 and June 30, 2002, the 23-valent polysaccharide pneumococcal vaccine (Pneumovax® 23; Merck & Co, Inc, Whitehouse Station, NJ, USA) was administered to HIV-infected patients after their informed consent was obtained. All patients were informed of the updated results of clinical studies regarding pneumococcal vaccination among HIV-infected patients. Patients who agreed to be vaccinated (recipients) and those who refused to be vaccinated (non-recipients) received the same standards of HIV medical care. The study was approved by the Institutional Review Board of the hospital.

Determinations of plasma human immunodeficiency virus RNA load and CD4 count

Plasma HIV RNA load (PVL) was quantified using real-time polymerase chain reaction (RT-PCR; Amplicor, version 1.5; Roche Diagnostics, Branchburg, NJ, USA) with a detection limit of 400 copies/mL (2.60 $\log_{10}$). CD4 counts were determined using FACFlow (Becton Dickinson). In those patients who were newly diagnosed as having HIV infection, baseline laboratory data were defined by values obtained within 1 month of the date of enrolment.

Streptococcus pneumoniae isolation and identification

Swab samples of the pharynx and tonsils were maintained at room temperature, transported to the laboratory, and plated on solid media within 24 h. The swabs were plated on sheep blood agar plates (BBL Microbiology System, Cockeysville, MD, USA) and incubated at 35°C with 5% carbon dioxide overnight. For each patient, 3 α-hemolytic colonies suspected to be *S. pneumoniae* were checked separately for optochin (Taxo P disc, BBL Microbiology System) susceptibility following the manufacturer's instructions. After overnight incubation at 35°C, bile solubility was performed on the colonies from the growth on the optochin plate following the standard microbiology protocol. Isolates that were both optochin susceptible and bile soluble were reported as *S. pneumoniae.* Isolates that were optochin resistant but bile soluble were resolved using the MicroSeq 16S rDNA microbial identification system (Applied Biosystems, Foster City, CA, USA). There were no optochin-susceptible bile-insoluble isolates.

Antimicrobial susceptibility testing

Minimal inhibitory concentrations (MICs) were determined using the broth microdilution method following the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [13] using standard hepatic, pancreatic, and biliary tract panels (Sensititre, Trek Diagnostic Systems Ltd, East Grinstead, UK). Quality control was performed on each day of the test with *S. pneumoniae* American Type Culture Collection (ATCC) 49619, *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Hemophilus influenzae* ATCC 49247. Penicillin susceptibility was defined according to the latest revised interpretative criteria of non-meningitis isolates as susceptible when the MIC was ≤2.0 μg/mL, intermediate when the MIC was 4.0 μg/mL, and fully resistant when the MIC was ≥8.0 μg/mL [14].

Streptococcus pneumoniae serotyping

Serotyping was performed following the manufacturer's instructions (Statens Serum Institut, Copenhagen, Denmark). Briefly, a bacterial suspension in phosphate buffered saline was prepared from the overnight blood agar plate. A drop each of the suspension was placed at 2 places on a glass slide, and a drop of the antiserum was added to 1 of the drops and mixed, then both drops were cover slipped. The mixture was examined under a phase contrast microscope to look for capsule by comparing the

antiserum-mixed drop with the control. The isolates were first checked using omni serum, which reacts with all 90 known *S. pneumoniae* types, followed by pooled sera, then factor sera. The pooled and factor sera used were suitable for the 23 serotypes present in the 23-valent polysaccharide pneumococcal vaccine only.

Definitions

Pneumococcal disease was defined as isolation of *S. pneumoniae* from a normally sterile site, or from a sputum or bronchial specimen in a patient with clinical symptoms and radiographic findings of pneumonia.

Statistical analysis

All statistical analyses were performed using the SAS statistical software (Version 8.1; SAS Institute Inc., Cary, NC, USA). Categorical variables were compared using chi-squared or Fisher's exact test, and non-categorical variables were compared using Wilcoxon rank sum test. The Cox-proportional hazards model was used to assess the effect of the pneumococcal vaccination on *S. pneumoniae* colonization with adjustment for age, sex, HIV transmission route, smoking, living with children younger than 10 years, category of baseline CD4+ count ($<200 \times 10^6$/L, $200\text{-}350 \times 10^6$/L, $\geq 350 \times 10^6$/L), PVL ≥ 5 $\log_{10}$ copies/mL, baseline acquired immunodeficiency syndrome–defining opportunistic illnesses [15], use of antimicrobials with potential activities against *S. pneumoniae*, such as penicillins, cephalosporins, fluoroquinolones, macrolides, and trimethoprim-sulfamethoxazole, hospital admission within 3 months of the culture being taken, and use of HAART. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for logistic regression analyses. All tests were 2-tailed. A p value of <0.05 was considered significant.

Results

The clinical characteristics of 302 HIV-infected patients who underwent swab cultures are shown in Table 1. Most of the patients acquired HIV through sexual routes; only 2.0% of the patients were injection drug users. The median baseline CD4 count was 155 cells/μL (range, 0-1226 cells/μL) when they first sought HIV care at the National Taiwan University Hospital. Nearly 80% of the patients were receiving HAART and their median CD4 count had increased to 250 cells/μL (range, 0-1259 cells/μL) when swab cultures were performed. Approximately 46% and 20% of the patients had received antibiotics or been admitted to hospital, respectively, within 3 months of the swab cultures (Table 1).

Twenty patients (6.6%) were colonized with *S. pneumoniae*: 15 of 198 patients who had received 23-valent polysaccharide pneumococcal vaccine (7.6%) and 5 of 104 who had not received vaccine (7.6%) [$p = 0.37$]. By univariate analysis, patients with *S. pneumoniae* colonization were more likely to be present or past smokers (76.9% vs 44.7%; $p = 0.05$) and to have pneumococcal disease before swab culture (10.0% vs 0.4%; $p = 0.01$). By multivariate analysis, smoking was the only significant predictive factor of *S. pneumoniae* colonization; the adjusted OR was 4.03 (95% CI, 1.04-15.64; $p = 0.04$). Patients with pneumococcal vaccination did not have a lower colonization rate than non-vaccinated patients (Table 1).

For the 198 patients (65.6%) who received 23-valent polysaccharide pneumococcal vaccine, the median interval between vaccination and swab culture was 420 days (range, 27-634 days). The characteristics of the vaccine recipients and non-recipients are shown in Table 2. Compared with non-recipients, the recipients had a higher proportion of acquisition of HIV infection through heterosexual transmission, and had significantly higher median CD4 count and better virologic suppression at the time of the swab culture. Subsequent follow-up revealed 1 episode of *S. pneumoniae* bacteremia in the vaccine recipients with elapse of 711 days from vaccination and 1 episode of pneumonia caused by *S. pneumoniae* in the non-vaccine recipients.

Of the 20 isolates of *S. pneumoniae*, 7 (46.7%) from the 15 vaccine recipients and 3 (60.0%) from the 5 non-vaccine recipients who had *S. pneumoniae* colonization were included in 23-valent polysaccharide pneumococcal vaccine serotypes; the serotype distribution did not differ significantly between the 2 groups (Table 3). Using the CLSI 2008 interpretative criteria for non-meningitis isolates, 17 isolates (85%) were penicillin susceptible and 3 (15%) were penicillin non-susceptible, 17 (85%) were resistant to clarithromycin, and 13 (65%) were resistant to trimethoprim-sulfamethoxazole. Previous antibiotic use was not associated with reduced susceptibility of *S. pneumoniae* isolates to penicillin and trimethoprim-sulfamethoxazole (Tables 4 and 5).

Discussion

In this study, the prevalence of *S. pneumoniae* colonization among HIV-infected patients was low (6.6%) compared

Table 1. Characteristics of patients with human immunodeficiency virus undergoing swab cultures of the pharynx and tonsils for *Streptococcus pneumoniae*.

Characteristic	Patients with *Streptococcus pneumoniae* colonization (A) No. (%)	Patients without *Streptococcus pneumoniae* colonization (B) No. (%)	All patients No. (%)	p^a
Patients	20 (6.6)	282 (93.4)	302	
Age (years) [median (range)]	39 (28-52)	37 (21-78)	37 (21-78)	0.93
Sex				
Male	19	259	278	1.00
Female	1	23	24	
Risk factor for HIV infection				
Heterosexual	4 (20.0)	104 (36.9)	108 (35.8)	0.21
Homosexual/bisexual	15 (75.0)	166 (58.9)	181 (59.9)	
Intravenous drug use	1 (5.0)	5 (1.8)	6 (2.0)	
Others	0 (0)	7 (2.5)	7 (2.3)	
CD4 at baseline (cells/μL) [median (range)]	112 (0-660)	156 (0-1226)	155 (0-1226)	0.46
<200	11 (61.1)	151 (58.1)	162 (58.3)	0.80
200-349	4 (22.2)	47 (18.1)	51 (18.3)	
≥350	3 (16.7)	62 (23.8)	65 (23.4)	
CD4 at swab culture (cells/μL) [median (range)]	248 (53-1259)	258 (0-1045)	250 (0-1259)	0.81
<200	8 (40.0)	99 (38.4)	107 (38.5)	0.40
200-349	8 (40.0)	72 (27.9)	80 (28.8)	
≥350	4 (20.0)	87 (33.7)	91 (32.7)	
PVL at baseline (log_{10} copies/mL) [median (range)]	4.82 (2.60-5.88)	4.51 (2.60-5.88)	4.57 (2.60-5.88)	0.17
≥5	8 (44.4)	108 (39.6)	116 (39.9)	0.81
<5	10 (55.6)	165 (60.4)	175 (60.1)	
<400	5 (27.8)	107 (39.2)	112 (38.5)	0.46
PVL at swab culture (log_{10} copies/mL) [median (range)]	2.60 (2.60-5.43)	2.60 (2.60-5.88)	2.60 (2.60-5.88)	0.56
≥5	2 (10.0)	24 (8.8)	26 (8.9)	0.70
<5	18 (90.0)	248 (91.2)	266 (91.1)	
<400	15 (75.0)	192 (70.6)	207 (70.9)	0.80
HAART at swab culture	16 (80.0)	223 (79.1)	239 (79.1)	1.00
Pneumococcal vaccination	15 (75.0)	183 (64.9)	198 (65.6)	0.37
Interval from vaccination to swab culture (days) [median (range)]	568 (171-628)	403 (27-634)	420 (27-634)	0.12
Interval ≥6 months	13 (92.9)	133 (72.2)	146 (73.8)	0.15
Smoking	10 (76.9)	80 (44.7)	90 (46.8)	0.05
Children at home	1 (7.7)	36 (20.0)	37 (19.3)	0.49
Antibiotic use at or before swab culture[b]	5 (25.0)	133 (47.2)	138 (45.7)	0.06
Hospital admission before swab culture	6 (30.0)	54 (19.1)	60 (19.9)	0.27
Hospital admission before swab culture due to respiratory tract disease	3 (15.0)	28 (9.9)	31 (11.0)	0.68
Subsequent pneumococcal disease	0 (0)	2 (0.7)	2 (0.7)	0.88
Pneumococcal disease before swab culture	2 (10.0)	1 (0.4)	3 (1.0)	0.01

[a] *p* values for A versus B.

[b] Antibiotics used included penicillins, cephalosporins, macrolides, fluoroquinolones, and trimethoprim-sulfamethoxazole.

Abbreviations: HIV = human immunodeficiency virus; HAART = highly active antiretroviral therapy; PVL = plasma human immunodeficiency virus RNA load.

with that of previous studies performed among HIV-infected adults or children in the pre-HAART era, regardless of pneumococcal vaccination [6-8,10,11]. Among HIV-infected children without HAART or pneumococcal vaccination, the colonization rate ranged from 19% to 86% [6-8]. Among HIV-infected adults not receiving antiretroviral therapy or pneumococcal vaccination, Janoff et al found that the rate

Table 2. Characteristics of patients with human immunodeficiency virus who did and did not receive vaccination with 23-valent capsular polysaccharide pneumococcal vaccine.

Characteristic	Vaccine recipients (n = 198) No. (%)	Non-vaccine recipients (n = 104) No. (%)	*p*
Age (years) [median (range)]	37 (21-78)	36 (21-64)	0.14
Sex ratio			0.38
Male	180	98	
Female	18	6	
Risk factor for HIV infection			
Heterosexual	81 (40.9)	27 (26.0)	0.03
Homosexual/bisexual	108 (54.5)	73 (70.2)	
Intravenous drug use	5 (2.5)	1 (1.0)	
Others	4 (2.0)	3 (2.9)	
CD4 at baseline (cells/μL) [median (range)]	176 (2-978)	154 (0-1226)	0.21
<200	107 (59.4)	55 (56.1)	0.28
200-349	36 (20.0)	15 (15.3)	
≥350	37 (20.6)	28 (28.6)	
CD4 at vaccination (cells/μL) [median (range)]	222 (2-1080)	NA	
<200	58 (41.4)		
200-349	46 (32.9)		
≥350	36 (25.7)		
CD4 at swab culture (cells/μL) [median (range)]	297 (4-1259)	135 (0-960)	<0.001
<200	51 (26.7)	56 (64.4)	<0.001
200-349	66 (34.6)	14 (16.1)	
≥350	74 (38.7)	17 (19.5)	
PVL at baseline (log_{10} copies/mL) [median (range)]	4.43 (2.60-5.88)	4.37 (2.60-5.88)	0.92
≥5	77 (40.7)	39 (38.2)	0.71
<5	112 (59.3)	63 (61.8)	
<400	81 (42.9)	31 (30.4)	0.04
PVL at vaccination (log_{10} copies/mL) [median (range)]	2.60 (2.60-5.88)	NA	
≥5	6 (3.3)		
<5	174 (96.7)		
<400	132 (73.3)		
PVL at swab culture (log_{10} copies/mL) [median (range)]	2.60 (2.60-5.26)	3.34 (2.60-5.88)	<0.001
≥5	5 (2.6)	21 (21.2)	<0.001
<5	188 (97.4)	78 (78.8)	
<400	167 (86.5)	40 (40.4)	<0.001
HAART initiated	162 (81.8)	77 (74.0)	0.14
Streptococcus pneumoniae colonization	15 (7.6)	5 (4.8)	0.37
Subsequent pneumococcal disease	1 (0.5)	1 (1.0)	0.69
Subsequent pneumococcal bacteremia	1 (0.5)	0 (0)	0.66

Abbreviations: HIV = human immunodeficiency virus; PVL = plasma human immunodeficiency virus RNA load; HAART = highly active antiretroviral therapy; NA = not applicable.

of *S. pneumoniae* colonization was 14%, which was not significantly higher than that of HIV-uninfected controls (9%) [10]. In this study, the colonization rate did not differ between HIV-infected patients with and without HAART (6.7% vs 6.3%; $p = 0.97$) or patients with and without pneumococcal vaccination (7.6% vs 4.8%; $p = 0.37$). However, the nasal carriage rates of *S. pneumoniae* in HIV-uninfected adolescents and adults in Taiwan before the wide implementation of pneumococcal vaccination were recently reported to be as low as 1.4% and 0%, respectively [16]. Hence, the lower rate of *S. pneumoniae* colonization in this study may result from different prevalences of *S. pneumoniae* colonization in the population.

The prevalence of *S. pneumoniae* colonization was not lower in patients administered 23-valent polysaccharide pneumococcal vaccination, which is similar to the findings of Rodriguez-Barradas et al [11], who investigated the rate of *S. pneumoniae* colonization in HIV-infected patients with 2 categories of CD4

Table 3. Serotype distribution of colonizing *Streptococcus pneumoniae* among patients with human immunodeficiency virus who did and did not receive vaccination with 23-valent capsular polysaccharide pneumococcal vaccine.

Serotype	Vaccine recipients with *Streptococcus pneumoniae* colonization (n = 15) No. (%)	Non-vaccine recipients with *Streptococcus pneumoniae* colonization (n = 5) No. (%)	*p*
Vaccine serotype	7 (46.7)	3 (60.0)	0.65
3	2 (28.6)	0 (0)	
6B	1 (14.3)	2 (66.7)	
19F	2 (28.6)	1 (33.3)	
23F	2 (28.6)	0 (0)	
Non-vaccine serotype	8 (54.3)	2 (40.0)	
6A	1 (12.5)	0 (0)	
Not typeable	7 (87.5)	2 (100)	

Table 4. Relationship between penicillin susceptibility of *Streptococcus pneumoniae* isolates and previous antibiotic use among patients with human immunodeficiency virus with *Streptococcus pneumoniae* colonization.

Characteristic	Penicillin-susceptible *Streptococcus pneumoniae* (n = 17) No. (%)	Penicillin–non-susceptible *Streptococcus pneumoniae* (n = 3) No. (%)	*p*
Minimal inhibitory concentration of penicillin			
≤0.06	4 (23.5)	0 (0)	
0.12-1	13 (76.5)	0 (0)	
2	0	0 (0)	
4	0	3 (100)	
≥8	0 (0)	0 (0)	
Antibiotic use at or before swab culture			
Number of patients[a]	5 (29.4)		0.80
Antibiotics used			
Trimethoprim-sulfamethoxazole	3 (17.6)	0 (0)	
Penicillins	1 (5.9)	0 (0)	
Cephalosporins	1 (5.9)	0 (0)	
Fluoroquinolones	1 (5.9)	0 (0)	
Macrolides	1 (5.9)	0 (0)	

[a]Some patients received more than 1 antibiotic.

counts (≥200 cells/μL and <200 cells/μL) and in HIV-uninfected controls who received 23-valent polysaccharide pneumococcal vaccine or conjugated pneumococcalvaccine. The rates of *S. pneumoniae* colonization changed from 20% to 22% in HIV-infected patients with CD4 count ≥200 cells/μL and from 7% to 23% in patients with CD4 count <200 cells/μL 6 months after vaccination. In HIV-uninfected control patients, the colonization rate decreased from 10% to 0%. In this study, vaccine recipients had significantly higher CD4 counts and lower plasma viral loads at swab culture than non-vaccine recipients, which could be partly explained by more vaccine recipients receiving HAART, while no association was demonstrated between these 2 laboratory parameters and pneumococcal colonization in multivariate analysis. These results are consistent with a recent cross-sectional study examining risk factors for pneumococcal carriage among mostly unvaccinated HIV-infected individuals in Brazil, that found that CD4 count and plasma viral load were not significantly associated with pneumococcal carriage [12].

The results of the multivariate analysis were in agreement with previous studies [9,12], in that smoking was the most significant factor associated with *S. pneumoniae* colonization. A recent study that examined the phagocytic capacity of alveolar macrophages in HIV-infected patients concluded that, while HIV infection alone does not impair phagocytic function, if combined with cigarette smoking, a significant depression in the phagocytic capacity can be demonstrated [17]. Although cigarette use is a largely modifiable risk factor, nearly half of the study participants were smokers,

Table 5. Relationship between trimethoprim-sulfamethoxazole susceptibility of *Streptococcus pneumoniae* and previous antibiotic use among patients with human immunodeficiency virus with *Streptococcus pneumoniae* colonization.

Characteristic	Trimethoprim-sulfamethoxazole–susceptible *Streptococcus pneumoniae* (n = 7) No. (%)	Trimethoprim-sulfamethoxazole–non-susceptible *Streptococcus pneumoniae* (n = 13) No. (%)	*p*
Minimal inhibitory concentration of trimethoprim-sulfamethoxazole			
≤0.05	7 (23.5)	0 (0)	
1-2	0 (0)	2 (15.4)	
≥4	0 (0)	11 (84.6)	
Antibiotic use at or before swab culture			
Number of patients[a]	3 (42.9)	2 (15.4)	0.41
Antibiotics used			
Trimethoprim-sulfamethoxazole	2 (28.6)	1 (7.7)	
Penicillins	0 (0)	1 (7.7)	
Cephalosporins	1 (14.2)	0 (0)	
Fluoroquinolones	1 (14.2)	0 (0)	
Macrolides	1 (14.2)	0 (0)	

[a]Some patients received more than 1 antibiotic.

so smoking cessation needs to be more aggressively pursued for prevention of pneumococcal colonization and subsequent pneumococcal diseases in HIV-infected patients.

The fact that more vaccine recipients acquired HIV infection through the heterosexual route than non-vaccine recipients was unexplained in this study, but is consistent with a previously published study [4]. As all patients were informed of the updated results of clinical studies regarding pneumococcal vaccination, the disparity in the acceptance of vaccination between different risk groups probably results from disproportionate concerns about the vaccine (for example, fear of side effects and/or increasing viremia). Former intravenous drug use was reported to be an independent risk factor for pneumococcal colonization in one study [12]. Whether different sexual risk may impact *S. pneumoniae* colonization is doubtful. The results showed no association between risk factors of HIV infection and pneumococcal colonization by multivariate analysis.

A recent study by Nicoletti et al examining *S. pneumoniae* carriage among HIV-infected adults (31% with a CD4 count <200 cells/μL at performance of swab culture) demonstrated the benefit of HAART for ≥1 year in decreasing the rate of *S. pneumoniae* colonization [12]. The lack of benefit of HAART in this study, despite increases of CD4 counts, may be related to the fact that the patients had depleted CD4 counts when HIV infection was first diagnosed. The CD4 count remained <200 cells/μL in more than 40% of the patients after HAART. The recovery of immunity may be limited and rapid loss of antibodies after pneumococcal vaccination may occur [18]. Whether institution of pneumococcal vaccination in patients with higher baseline CD4 counts will have any significant impact on *S. pneumoniae* colonization deserves further study.

Pneumococcal colonization plays a key role in pneumococcal disease and pneumococcal spread. Part of the preventive strategy for pneumococcal disease focuses on decreasing nasopharyngeal colonization by vaccination to reduce horizontal spread among the vaccinated community, especially among children [19]. Recently, a cohort study of 260 HIV-infected and HIV-uninfected Zambian women demonstrated that HIV infection significantly increased the risk of pneumococcal colonization [20]. Increased pneumococcal colonization may partly explain the higher rates of invasive pneumococcal disease in HIV-infected patients. The 23-valent polysaccharide pneumococcal vaccine is currently recommended for HIV-infected patients by the US Centers for Disease Control and Prevention [21], but studies have shown controversial results for the benefit of pneumococcal vaccination in this population. Although retrospective studies from Europe and North America have shown that pneumococcal vaccination protects against pneumococcal disease among HIV-infected patients [2-5], the only randomized, double-blind, placebo-controlled trial that studied the efficacy of the 23-valent polysaccharide pneumococcal vaccine among HIV-infected patients in Uganda not only failed to demonstrate benefit from vaccination, but also suggested increased risk of all-cause pneumonia

in the group of vaccinated patients [22]. In this study, the benefits of pneumococcal vaccination for protection against invasive pneumococcal disease were not demonstrated (0% vs 0.7%; $p = 1.00$). The discrepancy between the Uganda study and other studies may be explained by the use of HAART. However, lack of efficacy of the 23-valent polysaccharide pneumococcal vaccine in the reduction of pneumococcal colonization in this population may also contribute to the conflicted results. The results of this study also found that the distribution of vaccine and non-vaccine serotypes of *S. pneumoniae* that colonizes the upper airway of HIV-infected patients did not differ significantly between vaccine recipients and non-vaccine recipients. A better strategy, such as more immunogenic and efficacious vaccines, is needed for prevention of pneumococcal colonization and invasive pneumococcal disease in HIV-infected adults.

There are several limitations to this study. First, sampling was performed using oropharyngeal swabs instead of nasopharyngeal swabs. Nasopharyngeal sampling is preferable to oropharyngeal sampling in children [23], but there are inconsistent results for studies that examined the sensitivity of oropharyngeal and nasopharyngeal swabs among adults [23-28]. In some studies, nasopharyngeal sampling was superior to oropharyngeal sampling for identifying *S. pneumoniae* colonization in adults [23-25], while other studies either did not demonstrate differences between the 2 methods [26] or found a clear advantage to oropharyngeal swabs [27,28]. However, none of the studies were conducted among HIV-infected adults. Therefore, the optimal sampling site for detecting *S. pneumoniae* colonization remains unclear for this population. Second, this was a single-center study and the number of patients with *S. pneumoniae* colonization was small. Finally, the patients were provided with 23-valent polysaccharide pneumococcal vaccine, and the results may not be generalized to pneumococcal conjugate vaccine. Pneumococcal conjugate vaccine has been demonstrated to decrease colonization by vaccine serotypes, but to increase colonization with non-vaccine serotypes in children [29]. The direct effect of pneumococcal conjugate vaccine on *S. pneumoniae* colonization among HIV-infected adults remains unknown. A strategy combining a prime 7-valent pneumococcal conjugate vaccine followed by a boost with the 23-valent polysaccharide vaccine has been shown to improve immunogenicity against *S. pneumoniae* polysaccharides in HIV-infected adults [30]. Further studies are needed to investigate the impact of conjugate vaccine alone or with a prime-boost strategy for *S. pneumoniae* colonization in HIV-infected adults.

In conclusion, this study found that, in HIV-infected patients, vaccination with a 23-valent polysaccharide pneumococcal vaccine was not associated with a lower prevalence of *S. pneumoniae* upper airway colonization, while smoking was associated with a higher rate of *S. pneumoniae* colonization. As *S. pneumoniae* colonization plays a crucial part in the pathogenesis and prevention of pneumococcal infections, a better vaccination strategy, in addition to smoking cessation, may be needed to reduce *S. pneumoniae* colonization in HIV-infected adults.

References

1. Nuroti JP, Butler JC, Gelling L, Kool JL, Reingold AL, Vugia DJ. Epidemiologic relationship between HIV and invasive pneumococcal disease in San Francisco County, California. Ann Intern Med. 2000;132:182-90.
2. Breiman RF, Keller DW, Phelan MA, Sniadack DH, Stephens DS, Rimland D, et al. Evaluation of effectiveness of the 23-valent pneumococcal capsular polysaccharide vaccine for HIV-infected patients. Arch Intern Med. 2000;160:2633-8.
3. Dworkin MS, Ward JW, Hanson DL, Jones JL, Kaplan JE. Adult and Adolescent Spectrum of HIV Disease Project. Pneumococcal disease among human immunodeficiency virus-infected persons: incidence, risk factors, and impact of vaccination. Clin Infect Dis. 2001;32:794-800.
4. Hung CC, Chen MY, Hsieh SM, Hsiao CF, Sheng WH, Chang SC. Clinical experience of the 23-valent capsular polysaccharide pneumococcal vaccination in HIV-1-infected patients receiving highly active antiretroviral therapy: a 2-year prospective observational study. Vaccine. 2004;22:2006-12.
5. Penaranda M, Falco V, Payeras A, Jordano Q, Curran A, Pareja A, et al. Effectiveness of polysaccharide pneumococcal vaccine in HIV-infected patients: a case-control study. Clin Infect Dis. 2007;45:e82-7.
6. Rusen ID, Fraser-Roberts L, Slaney L, Ombette J, Lovgren M, Datta P, et al. Nasopharyngeal pneumococcal colonization among Kenyan children: antibiotic resistance, strain types and associations with human immunodeficiency virus type 1 infection. Pediatric Infect Dis. 1997;16:656-62.
7. Leibovitz E, Dragomir C, Sfartz S, Porat N, Yagupsky P, Jica S, et al. Nasopharyngeal carriage of multidrug-resistant *Streptococcus pneumoniae* in institutionalized HIV-infected and HIV-negative children in northeastern Romania. Int J Infect Dis. 1999;3:211-5.
8. Polack FP, Flayhart DC, Zahurak ML, Dick JD, Willoughby RE. Colonization by *Streptococcus pneumoniae* in human

immunodeficiency virus-infected children. Pediatr Infect Dis. 2000;19:608-12.
9. Madhi SA, Adrian P, Kuwanda L, Cutland C, Albrich WC, Klugman KP. Long-term effect of pneumococcal conjugate vaccine on nasopharyngeal colonization by *Streptococcus pneumoniae* — and associated interactions with *Staphylococcus aureus* and *Haemophilus influenzae* colonization — in HIV-infected and HIV-uninfected children. J Infect Dis. 2007;196:1662-6.
10. Janoff EN, O'Brien J, Thompson P, Ehret J, Meiklejohn G, Duvall G, et al. *Streptococcus pneumoniae* colonization, bacteremia, and immune response among patients with human immunodeficiency virus infection. J Infect Dis. 1993; 167:49-56.
11. Rodriguez-Barradas MC, Tharapel RA, Groover JE, Giron KP, Lacke CE, Houston ED, et al. Colonization by *Streptococcus pneumoniae* among human immunodeficiency virus-infected adults: prevalence of antibiotic resistance, impact of immunization, and characterization by polymerase chain reaction with BOX primers of isolates from persistent *S. pneumoniae* carriers. J Infect Dis. 1997;175:590-7.
12. Nicoletti C, Cristina M, Brandileone C, Luiza M, Guerra S, Levin AS. Prevalence, serotypes, and risk factors for pneumococcal carriage among HIV-infected adults. Diagn Microbiol Infect Dis. 2007;57:259-65.
13. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. 5th ed. NCCLS document M7-A5. Wayne: National Committee for Clinical Laboratory Standards; 2000.
14. Performance standards for antimicrobial susceptibility testing. 18th informational supplement. CLSI document M100-S17. Wayne: Clinical and Laboratory Standards Institute; 2008.
15. Centers for Disease Control and Prevention. 1993 Revised classification system for HIV infection and expanded surveillance case definition for AIDS among adolescents and adults. MMWR Morb Mortal Wkly Rep. 1992;41(RR-17):1-19.
16. Chen CJ, Huang YC, Su LH, Lin TY. Nasal carriage of *Streptococcus pneumoniae* in healthy children and adults in northern Taiwan. Diagn Microbiol Infect Dis. 2007;59: 265-9.
17. Elssner A, Carter JE, Yunger TM, Wewers MD. HIV-1 infection does not impair human alveolar macrophage phagocytic function unless combined with cigarette smoking. Chest. 2004;125:1071-6.
18. Nielsen H, Kvinesdal R, Benfield L, Lundgren JD, Konradsen HB. Rapid loss of specific antibodies after pneumococcal vaccination in patients with human immunodeficiency virus-1 infection. Scand J Infect Dis. 1998;30:597-61.
19. Bogaert D, De Groot R, Hermans PW. *Streptococcus pneumoniae* colonisation: the key to pneumococcal disease. Lancet Infect Dis. 2004;4:144-54.
20. Gill CJ, Mwanakasale V, Fox MP, et al. Impact of human immunodeficiency virus infection on *Streptococcus pneumoniae* colonization and seroepidemiology among Zambian women. J Infect Dis. 2008;197:1000-5.
21. Kaplan JE, Masur H, Holmes KK. Guidelines for the prevention of opportunistic infections among HIV-infected persons — 2002. Recommendations of the U.S. Public Health Service and the Infectious Diseases Society of America. MMWR Recomm Rep. 2002;51(RR-8):1-55.
22. French N, Nakiyingi J, Carpenter LM, Lugada E, Watera C, Moi K, et al. 23-valent pneumococcal polysaccharide vaccine in HIV-1-infected Ugandan adults: double-blind, randomised and placebo controlled trial. Lancet. 2000;355: 2106-11.
23. Foy HM, Wentworth B, Kenny GE, Kloeck JM, Grayston JT. Pneumococcal isolations from patients with pneumonia and control subjects in a prepaid medical care group. Am Rev Respir Dis. 1975;111:595-603.
24. Watt JP, O'Brien KL, Katz S, Bronsdon MA, Elliott J, Dallas J, et al. Nasopharyngeal versus oropharyngeal sampling for detection of pneumococcal carriage in adults. J Clin Microbiol. 2004;42:4974-6.
25. Lieberman D, Shleyfer E, Castel H, Terry A, Harman-Boehm I, Delgado J, et al. Nasopharyngeal versus oropharyngeal sampling for isolation of potential respiratory pathogens in adults. J Clin Microbiol. 2006;44:525-8.
26. Greenberg D, Broides A, Blancovich I, Peled N, Givon-Lavi N, Dagan R. Relative importance of nasopharyngeal versus oropharyngeal sampling for isolation of *Streptococcus pneumoniae* and *Haemophilus influenzae* from healthy and sick individuals varies with age. J Clin Microbiol. 2004; 42:4604-9.
27. Hendley JO, Sande MA, Stewart PM, Gwaltney JM Jr. Spread of *Streptococcus pneumoniae* in families. I. Carriage rates and distribution of types. J Infect Dis. 1975;132:55-61.
28. Boersma WG, Lowenberg A, Holloway Y, Kuttschrutter H, Snijder JA, Koeter H. The role of antigen detection in pneumococcal carriers: a comparison between cultures and capsular antigen detection in upper respiratory secretions. Scand J Infect Dis. 1993;25:51-6.
29. Klugman KP. Efficacy of pneumococcal conjugate vaccines and their effect on carriage and antimicrobial resistance. Lancet Infect Dis. 2001;1:85-91.
30. Lesprit P, Pédrono G, Molina JM, Goujard C, Girard PM, Sarrazin N, et al. Immunological efficacy of a prime-boost pneumococcal vaccination in HIV-infected adults. AIDS. 2007;21:2425-34.