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Immunogenicity and safety of pneumococcal conjugate polysaccharide and free polysaccharide vaccines alone or combined in HIV-infected adults in Brazil

Yeh-Li Ho[a,*], Angela Pires Brandão[b], Maria Cristina de Cunto Brandileone[c], Marta Heloisa Lopes[d]

[a] Divisão de Moléstias Infecciosas e Parasitárias, Hospital das Clinicas da Faculdade de Medicina da USP, Brazil
[b] Instituto Adolfo Lutz, Centro de Bacteriologia, Núcleo de Tuberculose e Micobacterioses, Brazil
[c] Instituto Adolfo Lutz, Centro de Bacteriologia, Núcleo de Meningites, Pneumonias e Infecções Pneumocócicas, Brazil
[d] Faculdade de Medicina da USP, Departamento de Moléstias Infecciosas e Parasitárias, Brazil

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ABSTRACT

Background: Streptococcus pneumoniae is a leading cause of hospitalization in HIV-infected adults therefore pneumococcal vaccine is recommended. The ideal antipneumococcal vaccine and effective vaccination regimen remain controversial and needs further evaluation.
Methods: To assess the efficacy of pneumococcal vaccines alone and combined, a randomized, blinded clinical trial was conducted in Brazil with 331 HIV-patients aged 18–60, with CD4-T cell count ≥200 cells/mm^3. Two interventions 60 days apart were done in three schedules: 23-valent pneumococcal polysaccharide vaccine (PPV23)/placebo; 7-valent pneumococcal conjugate vaccine (PCV7)/placebo; and PCV7 plus PPV23. Safety and reactogenicity were evaluated, and immunogenicity was assessed by an IgG enzyme-linked immunosorbent assay to *S. pneumoniae* serotypes 6B, 9V and 14, performed at baseline, 60 and 180 days after first intervention. Comparison of immunogenicity was based on geometric mean concentration (GMC), percentages of individuals with serotype-specific IgG ≥ 0.35 μg/mL and ≥1.0 μg/mL and proportion of individuals with ≥4-fold increase in specific antibody concentrations for each serotype.
Results: Demographic and HIV conditions were similar, and both vaccines were well tolerated across vaccine groups. Significant increase in IgG-antibodies was observed to all serotypes evaluated. A greater proportion of PCV7 recipients reached and sustained IgG antibody concentrations at least four times as high as those at baseline, for serotypes 6B and 9V. A PPV23 dose after PCV7 did not enhance immunogenicity.
Conclusions: In this first trial conducted with HIV-infected immunologically stable adults in South America, both PPV23 and PCV7 were safe and immunogenic. Evidence suggesting PCV7 was more immunogenic than PPV23, as it elicited higher and persistent ≥4-fold increase of antibodies for 6B and 9V serotypes in a greater proportion of HIV-patients is noteworthy. Despite current recommendation of schedules combining PCV7 and PPV23, there is little evidence to support this practice and we did not observe benefits in this combination.

1. Introduction

Invasive pneumococcal disease (IPD), as others bacterial diseases and sepsis represent important causes of hospitalization in human immunodeficiency virus (HIV) patients in the era of the highly active antiretroviral treatment (HAART) [1,2]. The risk of IPD in HIV-infected persons is 23–60 times higher than in HIV-uninfected individuals [3,4], even in the HAART era [5], and the mortality is also higher for HIV-infected adults [3,4]. Therefore, strategies to reduce the burden of IPD are crucial.

The 23-valent pneumococcal polysaccharide vaccine (PPV23), although recommended for HIV-infected adults, presents lower immunogenicity and effectiveness than to HIV-uninfected persons. Clinical trials showed impaired antibody response to PPV23 in HIV-infected patients compared to healthy individuals [6]. Additionally, antibody responses may be CD4-T cell dependent [7,8], making PPV23 administration preferential in the early course of HIV infection, when CD4-T cell counts are ≥200 cells/mm^3 [9], or after immune restoration through HAART. According to a meta-analysis [10], PPV23 was not effective in reducing IPD-related mortality rates in adults, even in populations for whom PPV23 is currently

* Corresponding author at: Divisão de Moléstias Infecciosas e Parasitárias, Hospital das Clinicas da Faculdade de Medicina da USP, Avenida Dr. Enéas de Carvalho Aguiar, 255, 4° andar, São Paulo, São Paulo BR 05403-000, Brazil.
Tel.: +55-11-26616530.
E-mail address: hoyehli@yahoo.com.br (Y.-L. Ho).

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recommended, despite vaccine protection for presumptive pneumococcal pneumonia and for all-cause pneumonia. In HIV-infected adults, conflicting results about PPV23 efficacy against IPD, pneumococcal pneumonia and other bacterial pneumonia have also been observed [11–18].

The use of pneumococcal conjugate vaccine (PCV) in adults is still controversial [19,20]. Some clinical trials in nonimmunosuppressed adults revealed higher antibody responses to PCV than to PPV23 [21–23], while inferior antibody response to PCV in elderly individuals was observed [24]. In a clinical trial comparing PPV23 and PCV in HIV-infected adults [25], higher antibody responses were observed after PCV for serotypes 6B, 18C, and 23F, but not for 19F. In HIV-infected adults with CD4-T cells counts $\geq$200 cells/mm^3, no difference was found between PPV23 and PCV for serotypes 4, 6B, 9V, 14, and 23F [26]. In one of the few studies evaluating the effectiveness of PCV in HIV-infected adults [27], significant protection against recurrence of IPD in HIV-infected adults was observed, however, this protection has decreased dramatically over time, from 85% in the first year to 25% thereafter.

Combined PCV/PPV23 schedules have been tested for HIV-infected adults. Two PCV doses given a month apart, followed by PPV23 nine months later, elicited significant increment of antibody concentration [28]. Lesprit et al. [29] observed in HIV-adults with CD4-T cell counts of 200–500 cells/mm^3 higher immunological response to PCV followed by PPV23 compared to one dose of PPV23, while no difference was observed between two doses of PCV and PCV/PPV23 schedules. On the other hand, no antibody increase was seen in a study [26] using PCV followed by either conjugate or polysaccharide vaccine.

This study aims to compare antibody response to PPV23 and PCV7 alone and combined in HIV-infected adults.

2. Materials and methods

2.1. Study design

A randomized, blinded clinical trial to compare the immunogenicity of PPV23 and PCV7 given in different schedules to HIV-infected adults was performed. Patients received two vaccine doses given 60 days apart, according to three vaccination schedules, as follows: PPV23 and then placebo vaccine (group A); PCV7 followed by placebo vaccine (group B); and PCV7 followed by PPV23 (group C). To assess vaccine-induced immune responses, peripheral venous blood samples were collected just before each vaccine dose (at baseline and day 60), and on day 180 (120 days after second dose). Simultaneously, blood samples were collected to perform CD4-T cells count and HIV viral load tests.

Sample size calculations, with a significance level of 0.05, a power of 0.80, and 10% loss to follow-up resulted in 140 patients in each group. Allocation to groups was on 1:1:1 basis generated by a table of random numbers from Epi-Info version 6.04. Study investigators and laboratorial executors were blinded to group allocation.

2.2. Study population

HIV-infected outpatients were recruited at the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (HCFMUSP), São Paulo, Brazil, the largest public hospital in South America. Individuals aged 18–60, with HIV infection documented by enzyme-linked immunosorbent assay (ELISA) and Western-blot testing, and with CD4-T cell count $\geq$200 cells/mm^3 in two different occasions in the past six months were eligible. Exclusion criteria included: any acute febrile illness at the moment of vaccination, active AIDS-defining clinical condition, previous immunization with any of the pneumococcal vaccines, any systemic malignancy neoplasm, use of immunoglobulin within the last three months, current pregnancy and antecedent of allergy to any of the pneumococcal vaccine components.

The study was approved by the Ethics Committee at HCFMUSP (process number 047/04). The trial is registered in clinical trial register/database under the number RBR-3P7577. Written informed consent was obtained from all participants. Principles of Declaration of Helsinski were followed and the study was conducted adhering to good clinical practice guidelines.

After screening, individual interview was performed and medical record was reviewed to obtain demographic characteristics and information related to HIV infection.

2.3. Vaccine administration

PPV23 (Pneumovax; Aventis Pasteur) contained 25 μg/dose of *Streptococcus pneumoniae* serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, and 33F. PCV7 (Prevenar; Wyeth) contained per dose 2 μg of pneumococcal serotypes 4, 9V, 14, 18C, 19F, 23F and 4 μg of serotype 6B individually conjugated to diphtheria CRM197 protein. Placebo vaccine consisted of physiological saline solution. Doses of 0.5 mL were administered by research nurses as deltoid-intramuscular injections.

2.4. Immunogenicity

Pneumococcal serotypes 6B, 9V, and 14 were selected for immunogenicity studies based on IPD data in adults [30] and on pneumococcal carriage by HIV-infected adults living in São Paulo [31] at the time this study was designed. They were among the most frequently isolated both from IPD and carriage and are common to both vaccines.

Total IgG anticapsular antibody concentrations to serotypes 6B, 9V, and 14 were measured by ELISA, according to the World Health Organization protocol [32]. In order to avoid assay variability, same lot microtiter plates, capsular and C-polysaccharides, and enzyme-conjugated anti-human IgG were used throughout this study. Before the analysis, both optimal antigen coating concentration and enzyme-conjugated anti-human IgG dilution were determined. Serum samples, aliquoted and stored at −70 °C until assayed, were reacted with pneumococcal C-polysaccharide and 22F capsular polysaccharide to adsorb antibodies to common contaminants that might be present in the coating antigens. Every ELISA plate contained a pooled human serum sample from vaccinated individuals as quality control and eight duplicate dilutions of a calibration standard, 89-SF (not adsorbed with serotype 22F polysaccharide). All serum samples belonging to a patient were analyzed simultaneously on the same plate, in duplicate and in six serial dilutions.

Antibody concentrations were calculated by comparing test sera with 89-SF using a four-parameter curve fit program from ELISA software [33]. Results were expressed as μg/mL calculated on the basis of the assigned IgG values for 89-S reference serum [34,35]. Samples with antibody concentrations <0.1 μg/mL or >100 μg/mL were confirmed upon retesting.

2.5. Statistical analysis

The pre- and post-vaccine antibody concentrations against each serotype were log transformed to obtain normality, and the results expressed as geometric mean concentration (GMC).

The following parameters were calculated: (a) GMCs for serotypes 6B, 9V and 14; (b) the percentages of individuals with serotype-specific IgG $\geq$ 0.35 μg/mL and $\geq$1.0 μg/mL; (c) proportion

Table 1
Baseline characteristics of the study population at time of inclusion.

	Group A (*N* = 111)	Group B (*N* = 110)	Group C (*N* = 110)	*p*
Male, *n* (%)[a]	77 (69.5)	86 (78.2)	84 (76.4)	0.28
Age, mean ± SD[b]	40.16 ± 9.41	40.12 ± 8.51	41.55 ± 7.76	0.37
AIDS*, *n* (%)[a]	65 (58.6)	65 (59.1)	69 (62.7)	0.79
CD4-T cells, mean ± SD[c]	548 ± 304	545 ± 238	492 ± 269	0.07
HIV viral load <400 copies/mm^3, *n* (%)[a]	71 (64)	74 (67.3)	79 (72.5)	0.39
HAART, *n* (%)[a]	81 (73)	84 (76.4)	89 (80.9)	0.38

SD, standard deviation; AIDS, acquired immunodeficiency syndrome-defining clinical condition; HAART, highly active antiretroviral therapy.
[a] Chi-squared test.
[b] ANOVA.
[c] Kruskal–Wallis.

of individuals with ≥4-fold increase in antibody concentrations for each serotype.

The values of 0.35 μg/mL and 1.0 μg/mL chosen as cut-off points do not necessarily correspond to seroprotective levels. Despite 0.35 μg/mL has been defined as the correlate of protection against IPD in infant, no seroprotective level has yet been established for adults.

Microsoft Excel 2003 was used to enter all data and statistical analysis was performed using Epi-Info v3.5.1 and StatPlus Professional v5.8.4. Differences between groups in proportion of individuals with IgG ≥ 0.35 μg/mL, IgG ≥ 1.0 μg/mL, and ≥4-fold increase in antibody concentrations were determined by two-tailed Fisher exact and Pearson Chi-squared tests. Medians were compared using ANOVA or Kruskal–Wallis tests. The level of significance was set at $p \leq 0.05$, with 95% confidence intervals (CI).

3. Results

3.1. Study population

From October 2005 to May 2009, 331 patients were enrolled in the study (111 individuals to group A, 110 to group B and 110 to group C). Enrollment was interrupted because institution overloading prevented further admissions of recently diagnosed HIV-patients. There was no differential loss to follow-up among study groups (Fig. 1).

Mean age of patients was 40.6 years, 74.6% male, mean time of HIV diagnosis was 6.7 years, and 60.1% had previous AIDS-defining clinical condition. Mean of CD4-T cells count was 525 cells/mm^3 and 67.3% had HIV-viral load <400 copies/mm^3. HAART was in use by 76.6% of patients. The three groups were similar in demographic characteristics and HIV conditions (Table 1).

3.2. Immunogenicity

At baseline there were no significant differences among the three groups in GMCs of antipneumococcal antibodies or in the proportion of individuals with IgG antibody concentration ≥0.35 μg/mL or ≥1.0 μg/mL for all serotypes analyzed (Table 2).

Sixty days after the initial dose of either PPV23 (group A) or PCV7 (groups B and C), all study groups showed significant increases in GMCs of the antipneumococcal antibodies for all serotypes (Fig. 2). At day 180, the antibody levels decreased in all groups for all serotypes, though less markedly for serotypes 6B and 14 in recipients of a PCV7 dose and PPV23 booster 60 days later (group C) (Fig. 2).

The mean concentration of antipneumococcal antibodies and the proportion of individuals with antibody concentrations ≥0.35 μg/mL and ≥1.0 μg/mL 60 and 180 days post-vaccination were similar for the three serotypes analyzed. However, the proportion of individuals who achieved at least a 4-fold increase in antibody levels was higher to serotypes 6B and 9V in patients primed with PCV7 (groups B and C), in both 60 and 180 days (Table 2).

No statistical differences were observed after receipt of PCV7 alone (group B) compared with a PPV23 dose 60 days after PCV7 priming (group C) as measured by antipneumococcal antibody GMCs, proportion of individuals with IgG antibody concentrations ≥0.35 μg/mL or ≥1.0 μg/mL, or presenting ≥4-fold increases in antibody levels (Table 2).

3.3. Reactogenicity

All strategies were well tolerated. No severe local or systemic symptoms were reported. After first vaccination, no statistical difference was observed between PPV23 and PCV7 in terms of local reaction, as pain, redness and swelling at the injection site. However, higher proportion of individuals vaccinated with PCV7 reported systemic symptoms, including fever, myalgia and asthenia (Table 3). No statistical difference was observed between PPV23 given alone (group A) or as a booster dose (group C).

4. Discussion

The role of pneumococcal vaccination and the best antipneumococcal vaccine to be recommended in adults is still controversial [19,20]. Recently, a pneumococcal 13-valent conjugate vaccine (PCV13) was approved in the USA for people aged ≥50 years and the Advisory Committee on Immunization Practices of USA (ACIP) recommended routine use of PCV13 for adults aged ≥19 years with immunocompromising conditions [36]. However, many questions about the use of pneumococcal vaccines are still unclear [37]. Furthermore, different from infants, the protective specific antibody concentration in adults is not determined. Questions as the best condition and the ideal schedule of vaccination of HIV-infected adults, and the lower post-vaccination responses than by immunocompetent individuals are otherwise unresolved problems [38–41].

Despite only patients with CD4-T cell ≥200 cells/mm^3 have been enrolled, some studies observed no impact of CD4-T count on PPV23 immunogenicity and determined the potential influence of antiretroviral therapy and HIV-viral load at the moment of vaccination with protective effect [14–16]. In our trial, the majority of patients was in current antiretroviral therapy and had HIV-viral load <400 copies/mm^3.

Antibodies levels at baseline were high to the three serotypes, especially to serotype 14, what might in part explain the absence of significant differences after vaccination, in terms of frequency of individuals with antibody concentrations ≥0.35 μg/mL or ≥1.0 μg/mL. Therefore, analysis of immunogenicity based on these two parameters may be of little value for some populations and age groups unlikely to be naive to pneumococcal antigens.

The finding that the proportion of individuals who reached a ≥4-fold increase in antibodies levels 60 days from baseline was higher in recipients of PCV7 may imply superior immunogenicity

Table 2
Serum IgG antibodies responses to pneumococcal capsular polysaccharides serotypes 6B, 9V, and 14 from HIV-infected adults before and after pneumococcal vaccines given in three vaccine schedules.

Serotype	Serum sample	Mean IgG concentration[a], μg/mL ($CI_{95\%}$)			IgG concentration[a] ≥0.35 μg/mL, *N* (%)			IgG concentration[a] ≥1.0 μg/mL, *N* (%)			IgG fold increase[a] ≥4, *N* (%)		
		A	B	C	A	B	C	A	B	C	A	B	C
6B	Pre[b]	0.73 (0.56–0.94)	0.51 (0.39–0.65)	0.57 (0.44–0.74)	76 (68.5)	68 (62.4)	70 (63.6)	46 (41.4)	29 (26.6)	40 (36.4)	n/a	n/a	n/a
	60 days[c]	1.45[e] (1.04–2.01)	1.88[e] (1.35–2.64)	2.12[e] (1.49–3.01)	74 (74.0)	81 (81.0)	88 (83.8)	58 (58.0)	63 (63.0)	67 (63.8)	13 (13.0)	42 (42.0)	38 (36.2)[**]
	180 days[d]	1.02[f] (0.73–1.42)	1.24[f] (0.88–2.50)	1.76[f] (1.24–2.5)	67 (75.3)	74 (81.3)	76 (83.5)	47 (52.8)	45 (49.5)	54 (59.3)	7 (7.9)	27 (30.0)	27 (29.7)[**]
9V	Pre	0.55 (0.43–0.69)	0.43 (0.33–0.55)	0.47 (0.38–0.58)	75 (67.9)	59 (54.1)	64 (58.2)	29 (26.1)	32 (29.4)	25 (22.7)	n/a	n/a	n/a
	60 days	1.76[e] (1.34–2.31)	2.74[e] (2.00–3.77)	2.97[e] (2.18–4.03)	90 (90.0)	89 (89.0)	97 (92.4)	69 (69.0)	74 (74.0)	78 (74.3)	42 (42.0)	58 (58.0)	66 (62.9)[*]
	180 days	1.37[f] (1.03–1.82)	1.68[f] (1.22–2.31)	2.13[f] (1.58–2.88)	79 (88.8)	77 (84.6)	81 (89.0)	56 (62.9)	55 (60.4)	60 (65.9)	25 (28.1)	41 (45.6)	41 (45.1)[*]
14	Pre	1.82 (1.36–2.42)	1.15 (1.08–2.16)	1.40 (1.01–1.91)	95 (85.6)	86 (78.9)	84 (76.4)	70 (63.1)	64 (58.7)	60(54.4)	n/a	n/a	n/a
	60 days	8.94[e] (6.36–12.58)	12.36[e] (8.15–18.74)	8.01[e] (5.70–11.26)	96 (96.0)	89 (89.0)	101 (96.2)	92 (92.0)	88 (88.0)	92 (87.6)	46 (46.0)	62 (62.0)	54 (51.4)
	180 days	6.72[f] (4.65–9.72)	9.32[f] (6.04–14.38)	6.35[f] (4.36–9.26)	86 (96.6)	81 (89.0)	86 (94.5)	79 (88.8)	76 (83.5)	77 (84.6)	35 (39.3)	50 (55.6)	42 (46.2)

CI denotes confidence interval.
A denotes group A, comprising persons vaccinated with 23-valent pneumococcal polysaccharide vaccine (PPV23) and placebo 60 days apart.
B denotes group B, vaccinated with pneumococcal 7-valent conjugate vaccine (PCV7) and placebo 60 days apart.
C denotes group C, in which persons were vaccinated with PCV7 followed by PPV23 60 days apart; n/a denotes not applicable.
Differences in means of IgG investigated by analysis of variance; else, by Pearson's Chi-square.

[a] Concentrations calculated in geometric mean.
[b] Pre-vaccination, *N* = 111 (group A), 109 (group B, 1 sample accidentally lost), 110 (group C).
[c] 60 days after 1st vaccine, *N* = 100 (groups A and B, 2 samples accidentally lost), 105 (group C).
[d] 180 days after 1st vaccine, *N* = 89 (group A), 91 (group B), 91 (group C, 1 sample accidentally lost).
[e] $p < 0.05$ for pre-vaccination *vs* 60 days.
[f] $p < 0.05$ for pre-vaccination *vs* 180 days.
[*] $p < 0.05$ for group A *vs* groups B and C.
[**] $p < 0.001$ for group A *vs* groups B and C.

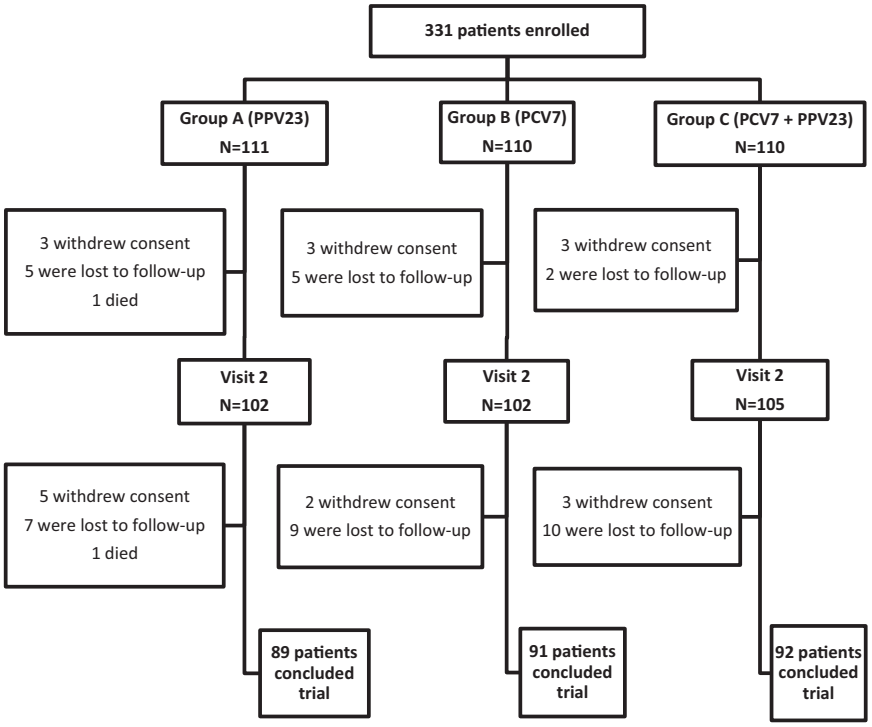

Fig. 1. Enrollment and follow-up visits. Group A: vaccinated with 23-valent pneumococcal polysaccharide vaccine and placebo 60 days apart. Group B: vaccinated with 7-valent pneumococcal conjugate vaccine and placebo 60 days after. Group C: vaccinated with 7-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine 60 days after.

induced by a single dose of PCV7 compared with PPV23. Our finding is in contrast with a recent study [42] using a different clinical trial design, in which 2-fold increases in antibody concentration between PCV7 and PPV23 were similar.

Decline in antibody concentrations 180 days after initial dose was similar and expectedly slight for all vaccine schedules. Interestingly again was the significant larger proportion of PCV7 recipients keeping higher serotypes 6B and 9V anticapsular antibody increments than those primed with PPV23. Consistent with previous reports [26,42] but in contrast with another study [29], we observed no benefit of booster with PPV23 after priming with PCV7.

Similar to previous studies [26,28,29], both vaccines were well tolerated. Systemic symptoms were more frequent after receipt of PCV7 than of PPV23, as observed in prior study [42], but in contrast with other [26]. Previous exposition to PCV7 did not alter the reactogenicity to PPV23, as reported earlier [28].

Limitations of our study comprise the narrow subset of vaccine serotypes evaluated, though they represent the most

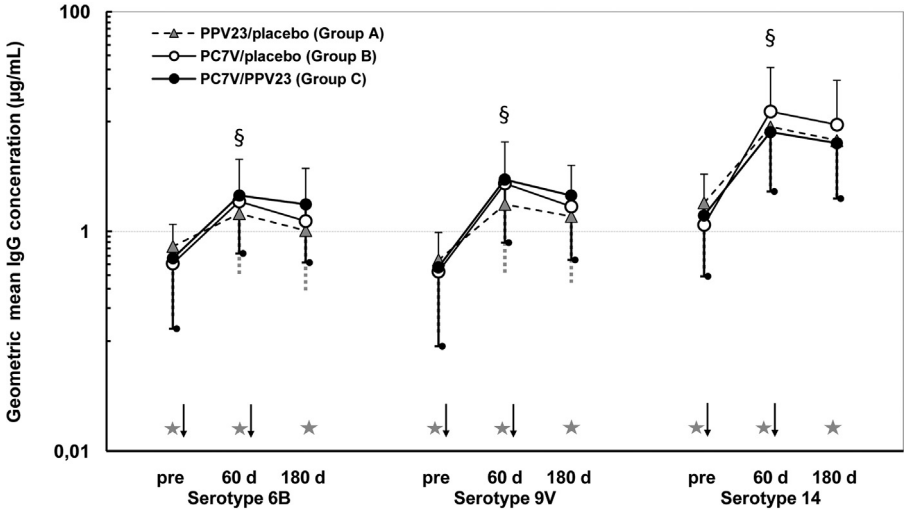

Fig. 2. Mean concentration and 95% confidence intervals (CIs) of serum IgG antibodies responses to capsular *Streptococcus pneumoniae* serotypes 6B, 9V, and 14 from HIV-infected adults before (pre), 60 days (60 d), and 180 days (180 d) after initial pneumococcal vaccine doses, given in three vaccination schedules (groups A, B and C). Dashed error bars are CIs for group A, solid bar ending with an horizontal dash, CI for group B and error bars ending with closed circles, CIs for group C; arrows indicate times of first (pre) and second (60 d) injection; stars, times of blood collection; §, significant higher geometric mean concentrations ($p < 0.05$) than at pre (baseline) for each group per analysis of variance.

Table 3
Subjects reporting local reactions or systemic symptoms.

	Local reaction, *n* (%)	Systemic symptoms, *n* (%)
Prime PPV23 *vs* PCV7		
PPV23	43 (39.1)	27 (24.5)
PCV7	90 (41.3)	77 (35.3)
Prime PPV23 *vs* PPV23 after PCV7		
PPV23	43 (39.1)	27 (24.5)
PPV23 after PCV7	29 (28.3)	18 (17.5)

PPV23, 23-valent pneumococcal polysaccharide vaccine; PCV7, pneumococcal 7-valent conjugate vaccine.

common serotypes causing IPD in adults in Brazil [30]. Evaluating vaccine-induced antibodies to more serotypes would add further information on the immunogenicity elicited by different vaccine schedules. Functional antibody activity, as measured by opsonophagocytic assay, was not assessed in our trial, however, previous studies have demonstrated that adsorbing test sera with pneumococcal 22F capsular polysaccharide improves correlation of IgG-ELISA with opsonophagocytic activity [43–45].

A major problem in immunogenicity trials in adults is the lack of a defined level of antibodies that correlates with disease prevention. Although IgG antibody levels $\geq$1.0 μg/mL for the majority of serotypes is required for protection in children [46], this is indefinite in adults, and the best method to determine post-vaccine serological turning is still controversial.

In conclusion, this first pneumococcal vaccine trial in HIV-infected adults immunologically stable conducted in South America showed both PPV23 and PCV7 elicited significant increase of IgG antibodies to serotypes 6B, 9V, and 14. PCV7 provided higher proportion of individuals who reached 4-fold increase of IgG and sustained this condition to serotypes 6B and 9V. Despite current recommendation of schedules combining PCV7 and PPV23 [38], there is little evidence to support this practice and we did not observe benefit in this combination. Both vaccines were well tolerated in HIV-infected adults.

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References

[1] Chiang HHHC, Lee CM, Chen HY, Chen MY, Sheng WH, Hsieh SM, et al. Admissions to intensive care unit of HIV-infected patients in the era of highly active antiretroviral therapy: etiology and prognostic factors. Crit Care 2011;14:R202.
[2] Akgun KM, Pisani M, Crothers K. The changing epidemiology of HIV-infected patients in the intensive care unit. J Intensive Care Med 2011;26:151–64.
[3] Kourtis AP, Ellington S, Bansil P, Jamieson DJ, Posner SF. Hospitalizations for invasive pneumococcal disease among HIV-1-infected adolescents and adults in the United States in the era of highly active antiretroviral therapy and the conjugate pneumococcal vaccine. J Acquir Immune Defic Syndr 2010;55:128–31.
[4] Cohen AL, Harrison LH, Farley MM, Reingold AL, Hadler J, Schaffner W, et al. Prevention of invasive pneumococcal disease among HIV-infected adults in the era of childhood pneumococcal immunization. AIDS 2010;24:2253–62.
[5] Jordano Q, Falcó V, Almirante B, Planes AM, del Valle O, Ribera E, et al. Invasive pneumococcal disease in patients infected with HIV: still a threat in the era of highly active antiretroviral therapy. Clin Infect Dis 2004;38:1623–8.
[6] Mascart-Lemone F, Gerard M, Libin M, Crusiaux A, Franchioly P, Lambrechts A, et al. Differential effect of human immunodeficiency virus infection on the IgA and IgG antibody responses to pneumococcal vaccine. J Infect Dis 1995;172:1253–60.
[7] Falco V, Jordano Q, Cruz MJ, Len O, Ribera E, Campins M, et al. Serological response to pneumococcal vaccination in HAART-treated HIV-infected patients: one year follow-up study. Vaccine 2006;24:2567–74.
[8] Dworkin MS, Ward JW, Hanson DL, Jones JL, Kaplan JE. Pneumococcal disease among human immunodeficiency virus-infected persons: incidence, risk factors, and impact of vaccination. Clin Infect Dis 2001;32:794–800.
[9] Center for Disease Control and Prevention. Guidelines for prevention and treatment of opportunistic infections in adults and adolescents. MMWR 2009;58:33.
[10] Braido F, Bellotti M, De Maria A, Cazzola M, Canonica GW. The role of pneumococcal vaccine. Pulm Pharmacol Ther 2008;21:608–15.
[11] 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.
[12] 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 prospective observational study. Vaccine 2004;22:2006–12.
[13] Lopez-Palomo C, Martin-Zamorano M, Benitez E, Fernandez-Gutierrez C, Guerrero F, Rodriguez-Iglesias M, et al. Pneumonia in HIV-infected patients in the HAART era: incidence, risk, and impact of the pneumococcal vaccination. J Med Virol 2004;72:517–24.
[14] 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.
[15] Teshale EH, Hanson D, Flannery B, Phares C, Wolfe M, Schuchat A, et al. Effectiveness of 23-valent polysaccharide pneumococcal vaccine on pneumonia in HIV-infected adults in the United States, 1998–2003. Vaccine 2008;26(46):5830–4.
[16] Rodriguez-Barradas M. Impact of pneumococcal vaccination on the incidence of pneumonia by HIV infection status among patients enrolled in the veterans aging cohort 5-site study. Clin Infect Dis 2008;46:1093–100.
[17] Pedersen RH, Lohse N, Ostergaard L, Sogaard OS. The effectiveness of pneumococcal polysaccharide vaccination in HIV-infected adults: a systematic review. HIV Med 2011;12:323–33.
[18] French N, Moore M, Haikala R, Kayhty H, Gilks CF. A case–control study to investigate serological correlates of clinical failure of 23-valent pneumococcal polysaccharide vaccine in HIV-1-infected Ugandan adults. J Infect Dis 2004;190:707–12.
[19] Pitsiou GG, Kioumis IP. Pneumococcal vaccination in adults: does it really work. Respir Med 2011;105:1776–83.
[20] Musher DM, Sampath R, Rodriguez-Barradas MC. The potential role for protein-conjugate pneumococcal vaccine in adults: what is the supporting evidence. Clin Infect Dis 2011;52:633–40.
[21] de Roux A, Schmole-Thoma B, Siber GR, Hackell JG, Kuhnke A, Ahlers N, et al. Comparison of pneumococcal conjugate polysaccharide and free polysaccharide vaccines in elderly adults: conjugate vaccine elicits improved antibacterial immune responses and immunological memory. Clin Infect Dis 2008;46:1015–23.
[22] Jackson LA, Neuzil KM, Nahm MH, Whitney CG, Yu O, Nelson JC, et al. Immunogenicity of varying dosages of 7-valent pneumococcal polysaccharide–protein conjugate vaccine in seniors previously vaccinated with 23-valent pneumococcal polysaccharide vaccine. Vaccine 2007;25:4029–37.
[23] Goldblatt D, Southern J, Andrews N, Ashton L, Burbidge P, Woodgate S, et al. The immunogenicity of 7-valent pneumococcal conjugate vaccine versus 23-valent polysaccharide vaccine in adults aged 50–80 years. Clin Infect Dis 2009;49:1318–25.
[24] Lazarus R, Clutterbuck E, Yu LM, Bowman J, Bateman EA, Diggle L, et al. A randomized study comparing combined pneumococcal conjugate and polysaccharide vaccination schedules in adults. Clin Infect Dis 2011;52:736–42.
[25] Ahmed F, Steinhoff MC, Rodriguez-Barradas MC, Hamilton RG, Musher DM, Nelson KE. Effect of human immunodeficiency virus type 1 infection on the antibody response to a glycoprotein conjugate pneumococcal vaccine: results from a randomized trial. J Infect Dis 1996;173:83–90.
[26] Feikin DR, Elie CM, Goetz MB, Lennox JL, Carlone GM, Romero-Steiner S, et al. Randomized trial of the quantitative and functional antibody responses to a 7-valent pneumococcal conjugate vaccine and/or 23-valent polysaccharide vaccine among HIV-infected adults. Vaccine 2001;20:545–53.
[27] French N, Gordon SB, Mwalukomo T, White SA, Mwafulirwa G, Longwe H, et al. A trial of a 7-valent pneumococcal conjugate vaccine in HIV-infected adults. N Engl J Med 2010;362:812–22.
[28] Kroon FP, van Dissel JT, Ravensbergen E, Nibbering PH, van Furth R. Enhanced antibody response to pneumococcal polysaccharide vaccine after prior immunization with conjugate pneumococcal vaccine in HIV-infected adults. Vaccine 2000;19:886–94.
[29] Lesprit P, Pedrono 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 Nov 30;21(18):2425–34.
[30] PAHO/WHO. Informe Regional de SIREVA II, datos por país y por grupos de edad sobre las características de los aislamientos de *Streptococcus pneumoniae*, *Haemophilus influenzae* y *Neisseria meningitidis* en procesos invasores. Washington, DC: OPS; 2008. p. 37–46.

[31] Nicoletti C, Brandileone MC, Guerra ML, Levin AS. Prevalence, serotypes, and risk factors for pneumococcal carriage among HIV-infected adults. Diagn Microbiol Infect Dis 2007;57:259–65.
[32] World Health Organization. Training Manual for Enzyme-Linked Immunosorbent Assay for the Quantitation of *Streptococcus pneumoniae* Serotype Specific IgG (Pn PS ELISA) (WHO13). Geneva, Switzerland: World Health Organization; 2004 (accessed 02.09.08) http://www.vaccine.uab.edu/
[33] Plikaytis B, Holder P, Carlone G. Program ELISA for Windows, Version 2.15. Atlanta, GA, USA: Centers for Disease Control and Prevention; 2005.
[34] Quataert SA, Kirch CS, Wiedl LJ, Phipps DC, Strohmeyer S, Cimino CO, et al. Assignment of weight-based antibody units to a human antipneumococcal standard reference serum, lot 89-S. Clin Diagn Lab Immunol 1995;2:590–7.
[35] Concepcion N, Frasch CE. Evaluation of previously assigned antibody concentrations in pneumococcal polysaccharide reference serum 89SF by the method of cross-standardization. Clin Diagn Lab Immunol 1998;5:199–204.
[36] Center for Disease Control and Prevention. Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine for adults with immunocompromising conditions: recommendations of the advisory committee on immunization practices (ACIP). MMWR 2012;61:816–9.
[37] O'Brien KL. Pneumococcal conjugate vaccine, polysaccharide vaccine, or both for adults? We're not there yet. Clin Infect Dis 2009;49:1326–8.
[38] Subramaniam KS, Segal R, Lyles RH, Rodriguez-Barradas MC, Pirofski LA. Qualitative change in antibody responses of human immunodeficiency virus-infected individuals to pneumococcal capsular polysaccharide vaccination associated with highly active antiretroviral therapy. J Infect Dis 2003;187:758–68.
[39] Balmer P, Cant AJ, Borrow R. Anti-pneumococcal antibody titre measurement: what useful information does it yield? J Clin Pathol 2007;60:345–50.
[40] Madhi SA, Adrian P, Cotton MF, McIntyre JA, Jean-Philippe P, Meadows S, et al. Effect of HIV infection status and anti-retroviral treatment on quantitative and qualitative antibody responses to pneumococcal conjugate vaccine in infants. J Infect Dis 2010;202:355–61.
[41] Nunes MC, Madhi SA. Safety, immunogenicity and efficacy of pneumococcal conjugate vaccine in HIV-infected individuals. Hum Vaccin Immunother 2012;8:161–73.
[42] Penaranda M, Payeras A, Cambra A, Mila J, Riera M. Conjugate and polysaccharide pneumococcal vaccines do not improve initial response of the polysaccharide vaccine in HIV-infected adults. AIDS 2010;24:1226–8.
[43] Feikin DR, Elie CM, Goetz MB, Lennox JL, Carlone GM, Romero-Steiner S, et al. Specificity of the antibody response to the pneumococcal polysaccharide and conjugate vaccines in human immunodeficiency virus-infected adults. Clin Diagn Lab Immunol 2004;11:137–41.
[44] Henckaerts I, Goldblatt D, Ashton L, Poolman J. Critical differences between pneumococcal polysaccharide enzyme-linked immunosorbent assays with and without 22F inhibition at low antibody concentrations in pediatric sera. Clin Vaccine Immunol 2006;13:356–60.
[45] Concepcion NF, Frasch CE. Pneumococcal type 22f polysaccharide absorption improves the specificity of a pneumococcal-polysaccharide enzyme-linked immunosorbent assay. Clin Diagn Lab Immunol 2001;8:266–72.
[46] Esposito S, Droghetti R, Faelli N, Lastrico A, Tagliabue C, Cesati L, et al. Serum concentrations of pneumococcal anticapsular antibodies in children with pneumonia associated with *Streptococcus pneumonia* infection. Clin Infect Dis 2003;37:1261–4.