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Safety and immunogenicity of the Cuban heptavalent pneumococcal conjugate vaccine in healthy infants. Results from a double-blind randomized control trial Phase I

Carlos P. Dotres Martínez [a], Nivaldo Linares-Pérez [b,*], María E. Toledo-Romaní [c], Yarisset Ricardo Delgado [d], Rinaldo Puga Gómez [a], Beatriz Paredes Moreno [b], Laura M. Rodriguez-Noda [b], Mayelín Mirabal Sosa [b], Darielys Santana Mederos [b], Dagmar García-Rivera [b], Yury Valdés-Balbín [b], David Goldblatt [e], Vicente Vérez-Bencomo [b],
Havana-Pneumococci Clinical Group, Carmen R. Ruiz [a], Yarmila García [a], Rafael del Valle-Rodríguez [a], Ada Rodríguez-Concepción [a], Dania Vega [a], María E. Mesa [a], José A. Broche [a]
Finlay-Pneumococci Project, Amarilis Pérez [b], Vladimir Echemendía [b], Marisel Martínez [b], Pablo Cabrera [b], Nayibi Nuñez [b], Annaliet Iglesias [b], Yanet Rodríguez [b], Nadezhda González [b], Marlene Armesto [b], Alina Álvarez [b]

[a] Children University Hospital "Juan Manuel Márquez", Marianao, Havana 11400, Cuba
[b] Finlay Vaccine Institute, 200 and 21 Street, Playa, Havana 11600, Cuba
[c] Tropical Medicine Institute "Pedro Kourí", Havana, Cuba
[d] University Hospital "Juan Manuel Márquez", Marianao, Havana, Cuba
[e] World Health Organization (WHO) Pneumococcal Serology Reference Laboratory, University College London, Institute of Child Health, London, United Kingdom

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ABSTRACT

Background: Cuba has a new pneumococcal conjugate vaccine candidate (PCV7-TT). This study evaluates the safety and immunogenicity in healthy infants using 2p+1 vaccination schedule.
Methods: A phase I, controlled, randomized and double blind clinical trial was designed. 30 unvaccinated healthy infants were included. 20 subjects were assigned to study group (PCV7-TT) and 10 to control group (Synflorix®) to receive the vaccines at 7, 8 months of age (primary series) and 11 months (booster dose). Blood samples were collected 30 days after second dose and post booster for antibodies measure analysis by ELISA and OPA. The statistics analysis included the frequency of occurrence for adverse events and the immune response. Non-parametric tests were used to compare the immune response. The clinical trial was published in the Cuban Public Register of Clinical Trials with code RPCEC00000173 available at http://registroclinico.sld.cu.
Results: Overall, the safety profile of PCV7-TT was similar to Synflorix®. Local reactions were predominant and systemic events were mild in severity. Swelling and redness were frequently associated with PCV7-TT mainly after the first dose (50% and 40% respectively). 15% and 10% of subject reported severe swelling after first dose with PCV7-TT and after second dose with Synflorix®. Mild fever (≥38–≤39), vomiting and sleep disturb were the systemic events reported. 100% of infants achieved pneumococcal IgG antibody concentrations ≥0.35 μg/ml after booster dose for serotypes 1, 14, 18C and 19F in each vaccine group. For serotypes 5, 6B and 23F, more than 80% infants vaccinated with Synflorix® or PCV7-TT

* Corresponding author at: Finlay Vaccine Institute, Clinical Research and Impact Evaluation Division, Dirección: Ave 21 #19810 e/198 y 200, Atabey, Playa, P.O. Box 16042, La Habana CP: 11600, Cuba.
E-mail addresses: carlos.dotres@nauta.cu (C.P.D. Martínez), nlinares@finlay.edu.cu (N. Linares-Pérez), mariaeugenia@ipk.edu.cu (M.E. Toledo-Romaní), yariricardo@infomed.sld.cu (Y.R. Delgado), puga@infomed.sld.cu (R.P. Gómez), bparedes@finlay.edu.cu (B.P. Moreno), lmrodriguez@finlay.edu.cu (L.M. Rodriguez-Noda), mmirabal@finlay.edu.cu (M.M. Sosa), dsantana@finlay.edu.cu (D.S. Mederos), dagarcia@finlay.edu.cu (D. García-Rivera), yvbalbin@finlay.edu.cu (Y. Valdés-Balbín), d.goldblatt@ucl.ac.uk (D. Goldblatt), vicente.verez@finlay.edu.cu (V. Vérez-Bencomo).

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achieved protective IgG GMC $\geq$ 0.35 μg/ml after booster dose. OPA proportion's responders to the seven common serotypes were 89.5% or more after the primary dose and 100% after booster dose in vaccinated with PCV7-TT.
Conclusions: The Cuban PCV7-TT is safe, well tolerated and immunogenic in healthy infants.

1. Introduction

Streptococcus pneumoniae is responsible for pneumococcal invasive disease (IPD), causing significant morbidity and mortality worldwide [1]. Prevention of pneumococcal infection through vaccination remains the best strategy to reduce the incidence of IPD. The pneumococcal conjugate vaccines (PCVs) have been shown to be highly immunogenic, safe, well tolerated and effective in reducing invasive and noninvasive pneumococcal diseases in vaccinated children [2]. Besides their direct effects in infants, PCVs have a substantial indirect effects, resulting in the reduction of disease burden among unvaccinated adults and toddlers [3,4]. Available evidences from various countries have shown that PCVs can be administrated concurrently with other vaccines that are typically recommended during the first year of life [5,6].

Baseline data in Cuba previous 2014 about pneumococcal serotype distribution is really limited and based mainly in meningeal isolations. The first study exploring the prevalence of NP colonization in children was conducted and published in 2017 [7].

To date Cuba and many other low-income countries have not yet introduced pneumococcal vaccination into their national immunization programs due to their elevated costs. Indeed, despite significant global efforts to facilitate vaccine procurement through different advantageous financial mechanisms, the introduction of these complex and relatively expensive vaccines has been lengthy worldwide [8]. In 2007, Cuba initiated the development of a seven-valent tetanus toxoid conjugated PCV (PCV7-TT), the first in a subsequent series of more complex PCVs. PCV7-TT was developed bearing in mind the following criteria: (a) to include seven serotypes, learning from Prevnar-7 that the impact of PCV could be high if the selected serotypes match with the current epidemiology; (b) to use tetanus toxoid conjugates to increase the immunogenicity induced against the serogroups 19 and 6 through 19F and 6B conjugates; and (c) to reduce the time of pharmaceutical development by reducing the complexity of the vaccine and making it available as soon as possible. The final composition of this vaccine consists of 2 μg of PS from serotypes 1, 5, 14, 18C, 19F, 23F and 4 μg of 6B, all conjugated to tetanus toxoid and adsorbed on aluminum phosphate [9]. The vaccine is currently in late clinical development [10,11] before the introduction in Cuba in 2019. The seven serotypes contained in the new vaccine candidate representing 49.9% of total IPD in Cuba in 2009 [12] and 42.3% in 2014 [13]. Expected potential cross protection effect for 6A and 19A, the serotype coverage will raises more than 70%.

We expect that the Cuban PCV7-TT may represent an appealing option for countries that have not yet introduced PCV considering its price. Indeed, the seven prevalent serotypes included in the vaccine account for over 60% of isolated serotypes worldwide [14]. In the present article, we provide the first evidences of safety and immunogenicity of PCV7-TT in infants aged 7–11 months, who have not been previously vaccinated against pneumococcal diseases.

2. Methods

2.1. Study design and ethical considerations

A phase I, parallel, controlled, randomized and double blind clinical trial was designed with the primary objective to assess the safety of PCV7-TT in infants, using a two primary doses plus a booster (2p+1) schedule administrated at 7, 8 and 11 months of age. The secondary objective was to evaluate the immunogenicity of the PCV7-TT among the same study population.

The study protocol was reviewed and approved by the Ethics Committee of the Children University Hospital "Juan Manuel Márquez" in Havana, Cuba. The clinical trial was authorized by the Cuban National Regulatory Agency according the Good Clinical Practice (GCP) Cuban guidance [15], and published in the Cuban Public Register of Clinical Trials with the code RPCEC00000173 (a protocol summary is available at http://registroclinico.sld.cu). It was conducted in accordance with the code of Ethic of the World Medical Association for experiments in human beings [16].

2.2. Informed consent

Written informed consent was obtained from the parents or legally acceptable representative of each infant before enrollment. Physicians explained in details the benefits and risk of the study and parent's voluntary decided about the enrollment of their infant.

2.3. Enrollment and selection criteria

As PCV7-TT had not been previously evaluated in this population, 30 healthy infants from 38 eligible infants were randomized to the study and control groups. They were 7-months-old residents of Havana, who had not been previously vaccinated against pneumococcal disease and were enrolled upon parents' informed consent.

2.4. Inclusion criteria

We included healthy 7-months-old infants born at least at 36 weeks of gestation, weighing more than 2500 g at birth, presenting adequate nutritional condition and having completed the vaccination schedule during their first semester of life.

2.5. Exclusion criteria

Infants were excluded if they had history of chronic diseases, immunomodulator treatment, any hypersensitivity reaction following a previous vaccination, prior vaccination against *S. pneumoniae*, or acute illness at the moment of vaccination. Infants were also excluded if another vaccine had been administered, or planned, from 30 days before and up to 30 days after the administration of a study vaccine dose.

2.6. Vaccines, target groups and schedule

Eligible participants were randomly assigned in a 2:1 ratio to receive PCV7-TT or Synflorix® at 7, 8 months of age (primary dose) and 11 months (booster dose), based on a random assignment schedule prepared by the sponsor. All participants, study staff, and those assessing the outcomes were blinded to the group assignment. The intervals between consecutive primary vaccination doses were 30 days and 90 days between primary schedule and booster dose. We generated a random list using the R software

for Statistical Computing and a randomization of 2:1 to assign 20 subjects to the group PCV7-TT and 10 subjects to the group Synflorix®. At enrollment, each child was assigned to the study or control group as specified inside a sealed envelope with a sequential number. Blinded statistical analysis was conducted.

PCV7-TT (Batch NEU.13.02, Manufacturer: Finlay Vaccine Institute, Cuba) contains 2 μg of serotypes 1, 5, 14, 18C, 19F, 23F and 4 μg of serotype 6B, each one conjugated to TT for 24.5 μg of total TT in the formulation, 125 μg of aluminum phosphate and 0.026 mg of thiomersal. The vaccine is a suspension for injection and is presented in single dose vial; one dose is contained in 0.5 ml. Its routine storage is between 2 and 8 °C. Synflorix® vaccine (Batch ASPNA305AA, Manufacturer: GSK) was used as control. It is composed of the purified capsular polysaccharides from 10 serotypes 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F each one conjugated to a carrier protein, either protein D (PD), tetanus toxoid (TT) or diphtheria toxoid (DT). Synflorix® is a preservative-free liquid suspension, adsorbed on aluminum phosphate and presented as pre-filled glass syringes ready for intramuscular injection [17].

Both vaccines were administered intramuscularly with a 22–25 gauge needle (25 mm/long) into the right thigh of the infants. They received a 2-dose primary vaccination series (30 days interval) followed by booster vaccination (90 days interval) (Fig. 1), therefore, the schedule was at 7, 8 and 11 months of age. Blood specimen collection was scheduled at 30 days after the second and the booster dose.

In order to assure the double-blinding due to the different presentation of both vaccines, we used a double-nurse system where one nurse prepares the vaccine and the other vaccinates the child.

2.7. Safety and reactogenicity evaluation

The primary outcome of this study was the safety of the vaccine. Post-vaccination, children were followed up for 30 days to detect and assess any adverse event. Each subject was closely observed during three hours post-vaccination for identification of immediate adverse effects. Expected local reactions and systemic events were recorded actively during the 7 days post-vaccination period through medical visit. Unexpected adverse events information were collected during the 30 days post-vaccination period.

Diary cards were used to report the occurrence of local symptoms (pain, redness swelling) and general symptoms (i.e. fever, gastrointestinal symptoms, sleep disturbance) for 7 days after each doses. The occurrence of other (unsolicited) adverse events was also collected using diary cards for 30 days after each dose. Serious adverse events (SAEs) defined as events that were life-threatening, required hospitalization or prolong the hospitalization, or resulted in disability, incapacity or death, were recorded throughout the study. The investigators assessed the association between investigational products and the occurrence of each adverse event. Solicited local adverse events were all assumed to be associated with vaccine administration.

2.8. Immunogenicity evaluation

The secondary outcome of this study was to assess the vaccine's immunogenicity. Blood samples were collected 30 days after the second and the booster dose. Serum samples were stored at −20 °C until ELISA and OPA analysis. Three hundred microliters of each serum samples were analyzed for ELISA and OPA at the World Health Organization (WHO) Pneumococcal Serology Reference Laboratory, at the Institute of Child Health in London, the United Kingdom.

Pneumococcal serotype-specific anti-capsular polysaccharide antibodies for the seven serotypes included in PCV7-TT and for the additional serotypes 19A and 6A (contained only in 13-valent PCV, not in Synflorix® or PCV7-TT) were measured by ELISA using double absorption with C and 22F polysaccharides, following the WHO recommended protocol [18]. Functional opsonophagocytic antibodies were measured by viable counting MOPA method using differentiated HL60 cells as effector cells. The OPA titer was expressed as the reciprocal of the serum dilution that caused a 50% reduction of the colony-forming units compared to a control that contained no human serum, following the WHO recommended protocol [19]. Additionally, the antibody response to carrier protein TT was evaluated at the Finlay Vaccine Institute, using indirect ELISA assay for quantification of tetanus antitoxin in human serum, with the use of a previously calibrated standard [20].

2.9. Statistics analysis

The study corresponded to an exploratory phase I trial. Sample size was established by expert opinion and the analyses were performed on the Total Vaccinated Cohort.

2.10. Safety

We summarized the number and percentage of subjects with any solicited or general adverse events within the 7-day follow-up period per dose. We tabulated the number and percentage of subjects with unsolicited adverse events within the 30-day follow-up period according to the Medical Dictionary for Regulatory Activities (MedDRA) preferred terms. We tabulated SAEs occurring during the study period, including the long-term safety follow-up, according to MedDRA preferred terms. We used a non-parametric test (Fisher's Exact Test for Count Data, with error $\alpha = 0.05$ or 95% of safety) to compare incidence for each adverse event. We reported the frequency of occurrence for adverse events in both groups.

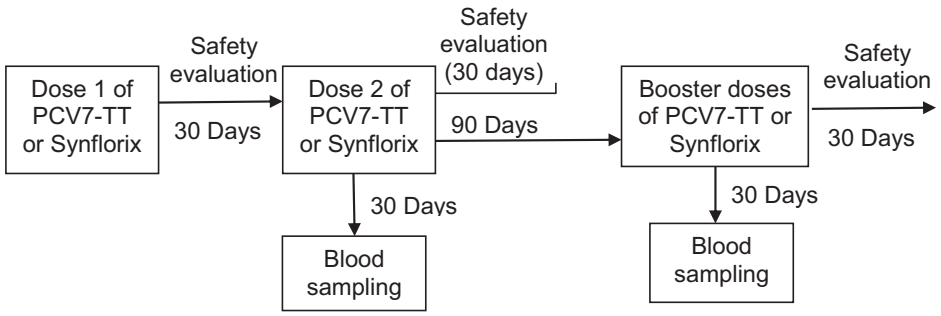

Fig. 1. Immunization and blood sampling schedule for infants included in the study. Safety was evaluated 30 days after each immunization. Blood samples were collected 30 days after primary immunization series and after booster dose.

2.11. Immunogenicity

We tabulated the number and proportion of subjects achieving a serotype-specific IgG antibody concentration ≥0.35 μg/ml (responders) measured one month after the primary series and booster for seven common serotypes. We calculated the exact 2-sided 95% confidence intervals (CIs) on the proportions. We calculated the geometric mean antibody concentrations (GMCs) with their 95% CI for each serotype and at each applicable blood specimen collection time point for both group. We estimated two-sided 95% CIs by back transformation of the CIs for the mean of the logarithmically transformed assay results. We reported the functional antibody activity (OPA) as a geometric mean titer with 2-sided 95% confidence intervals. Non-parametric tests were used to compare after the primary series and after the booster dose between groups for each serotype and for TT antibodies (Mann Whitney U Test and Sign test with error $\alpha = 0.05$). We calculated the number of subjects with OPA titer ≥1:8 for each serotype in both groups.

3. Results

From 38 subject screened, a total of 30 infants were enrolled and randomly assigned to each group: 29 infants completed the primary vaccination series and booster dose. Only one loss to follow up occurred (Fig. 2). Demographic characteristics of the two groups were similar with regards to gender, ethnicity and age.

3.1. Pneumococcal vaccine safety

Comparing infants in the two vaccine groups, in general, the safety profile of PCV7-TT was similar to that of Synflorix® (Table 1). Most of the local reactions and systemic events reported were mild. Local reactions after any dose were the most frequently reported and their duration ranged between three and eleven days. Swelling ($p = 0.048$) and redness at the injection site were more frequently associated with PCV7-TT mainly after the first dose (50% and 40% of vaccinated subject respectively). Fifteen percent and 10% of subjects respectively reported severe swelling at injection site following the first dose with PCV7-TT and after the second dose with Synflorix®. Mild fever (≥38–≤39 °C), vomiting and sleep disturbance were the systemic solicited events reported. No statistical differences were detected among the proportion of subject in both vaccinated groups with local or systemic adverse events. Fever was more frequent after the first dose of PCV7-TT (in 4/20 infants). The duration of systemic events did not last more than three days. The frequency of medication to treat symptoms was approximately 40% in each group after the first dose and ranged from 26% to 50% after the primary vaccination series and booster dose. Unsolicited events reported in infants vaccinated with PCV7-TT were diarrhea (5.15% per total applied dose) and rhinorrhea (3.45%). In the Synflorix® vaccinated group, diarrhea and rash were reported in 3.3% (per total applied dose), and hyperemic conjunctival in 6.6%. All these events were more frequent after the first and second dose. No deaths were reported in this study.

3.2. Pneumococcal vaccine immunogenicity

Table 2 shows the percentage of subjects who achieved a pneumococcal IgG antibody concentration ≥0.35 μg/ml after the primary series. All infants achieved a pneumococcal IgG antibody concentrations ≥0.35 μg/ml after the primary series for serotypes 1, 14 and 18C in each vaccine group. The percentages of infants achieving IgG antibody concentrations ≥0.35 μg/ml against serotype 19F were also high (94.7% in the PCV7-TT vaccinated group). 66.7% infants vaccinated with Synflorix® and 89.47% with PCV7-TT achieved protective IgG titers for 23F. More than 77% of vaccinated in both groups were responders for serotypes 5 and 6B. Regarding the IgG GMC, we found statistical difference among both groups only for serotypes 18C ($p = 0.000$) and 23F ($p = 0.003$). No

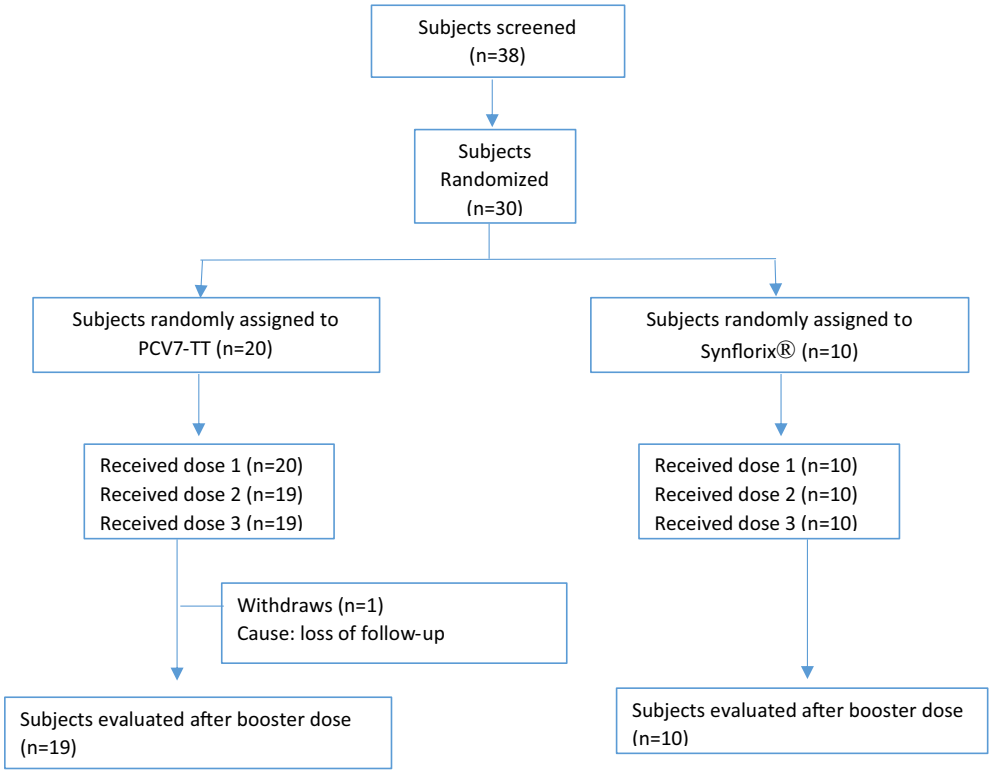

Fig. 2. Participant's flowchart.

Table 1
Proportion of subject reporting local or systemic events within 30 days after each dose of PCV7-TT or Synflorix® vaccine.

	Dose I			Dose II			Booster dose		
	PCV7-TT (N = 20)n (n/N)	Synflorix® (N = 10)n (n/N)	p value	PCV7-TT (N = 19)n (n/N)	Synflorix® (N = 10)n (n/N)	p value	PCV7-TT (N = 19)n (n/N)	Synflorix® (N = 10)n (n/N)	p value
Local reactions									
Tenderness									
Any	3 (15)	1 (10)	1	0 (0)	0 (0)	–	1 (5.26)	1 (10)	1
Moderate	0 (0)	1 (10)		0 (0)	0 (0)		0 (0)	0 (0)	
Redness									
Any	8 (40)	4 (40)	1	4 (21.05)	1 (10)	0.633	3 (15.79)	0 (0)	0.532
Swelling									
Any	10 (50)	1 (10)	0.048	5 (26.32)	2 (20)	1	3 (15.79)	1 (10)	1
Moderate	2 (10)	1 (10)		2 (10.53)	1 (10)		2 (10.53)	0 (0)	
Severe	3 (15)	0 (0)		2 (10.53)	0 (0)		0 (0)	0 (0)	
Increase local temperature									
Any	1 (5)	0 (0)	1	2 (10.53)	0 (0)	0.532	1 (5.26)	0 (0)	1
Total of subject with any local reactions	10 (50)	5 (50)	1	5	2	1	3	2	1
Systemic events									
Fever (°C) (≥38–<39)	4 (20)	0 (0)	0.268	2 (10.53)	1 (10)	1	1 (5.26)	2 (20)	0.267
Vomiting	1 (5)	0 (0)	1	1 (5.26)	1 (10)	1	0 (0)	0 (0)	–
Sleep disturb	0 (0)	1 (10)	0.345	0 (0)	0 (0)	–	0 (0)	0 (0)	–
Total of subject with any systemic events	4 (20)	1 (10)	0.633	3 (15.79)	2 (20)	1	1 (5.26)	2 (20)	0.267

Table 2
Comparison of IgG GMC response elicited by PCV7-TT and Synflorix® after primary infant series (two doses).

Serotypes	% responders			IgG GMC μg/ml		
	PCV7-TT% (95%CI) N = 19	Synflorix% (95%CI) N = 9	p-value	PCV7-TTGMC (95%CI) N = 19	SynflorixGMC (95%CI) N = 9	p-value
Vaccine serotypes						
1	100 (87–100)	100 (77–100)	1	1.893 (1.276–2.809)	2.636 (1.399–4.967)	0.328
5	78.9 (57–91)	88.9 (57–98)	1	0.643 (0.453–0.911)	1.091 (0.6–1.981)	0.089
6B	89.5 (69–97)	77.8 (45–94)	0.574	0.991 (0.654–1.502)	0.56 (0.254–1.234)	0.136
14	100 (87–100)	100 (77–100)	1	6.805 (4.277–10.827)	5.221 (3.025–9.01)	0.470
18C	100 (87–100)	100 (77–100)	1	3.455 (2.466–4.84)	8.024 (5.937–10.845)	0.0005
19F	94.7 (75–99)	100 (77–100)	1	2.908 (1.811–4.671)	4.581 (2.374–8.842)	0.246
23F	89.5 (69–97)	66.7 (35–88)	0.290	2.379 (1.368–4.139)	0.491 (0.186–1.294)	0.003
Vaccine related serotypes						
6A	52.6 (32–73)	33.3 (12–65)	0.435	0.289 (0.169–0.496)	0.218 (0.111–0.427)	0.509
19A	26.3 (12–49)	55.6 (27–81)	0.210	0.244 (0.177–0.335)	0.352 (0.158–0.787)	0.183

significant differences were detected for serotypes 6A and 19A among both groups neither for% of responders or IgG antibody concentration.

After booster dose (Table 3), 100% of infants achieved pneumococcal IgG antibody concentrations ≥0.35 μg/ml for serotypes 1, 14, 18C and 19F in each vaccine group. Also it was reach for serotypes 6B and 23F in vaccinated with PCV7-TT. For serotype 5, more than 80% infants vaccinated with Synflorix® or PCV7-TT achieved protective IgG GMC ≥ 0.35 μg/ml after booster dose. No statistical differences were detected for serotypes 6A (p = 0.08) and 19A

Table 3
Comparison of IgG GMC response elicited by PCV7-TT and Synflorix® after booster dose.

Serotypes	% responders			IgG GMC μg/ml		
	PCV7-TT% (95%CI) N = 19	Synflorix% (95%CI) N = 10	p-value	PCV7-TTGMC (95%CI) N = 19	SynflorixGMC (95%CI) N = 10	p-value
Vaccine serotypes						
1	100 (87–100)	100 (79–100)	1	1.42 (0.97–2.08)	2.599 (1.329–5.085)	0.077
5	94.7 (75–99)	90 (60–98)	1	0.898 (0.668–1.207)	1.026 (0.503–2.091)	0.804
6B	100 (87–100)	90 (60–98)	0.345	2.429 (1.655–3.563)	1.416 (0.532–3.767)	0.308
14	100 (87–100)	100 (79–100)	1	8.082 (6.123–10.668)	7.297 (4.474–11.901)	0.673
18C	100 (87–100)	100 (79–100)	1	2.368 (1.634–3.431)	7.954 (3.939–16.062)	0.001
19F	100 (87–100)	100 (79–100)	1	4.385 (2.679–7.177)	10.196 (5.855–17.757)	0.031
23F	100 (87–100)	80 (49–94)	0.111	2.722 (1.783–4.157)	1.065 (0.391–2.903)	0.069
Vaccine related serotypes						
6A	84.2 (62–94)	50 (24–76)	0.083	0.833 (0.459–1.513)	0.583 (0.227–1.494)	0.475
19A	63.2 (41–81)	100 (79–100)	0.065	0.536 (0.284–1.013)	1.55 (0.634–3.789)	0.046

Table 4
Booster effect after complete 2p+1 schedule. Comparison of IgG GMC after the primary series (two doses) with booster dose for PCV7-TT and Synflorix®.

Serotypes	PCV7-TT IgG GMC μg/ml			Synflorix® IgG GMC μg/ml		
	Primary series (two doses)GMC (95% CI) N = 19	BoosterGMC (95% CI) N = 19	p-value	Primary series (two doses)GMC (95% CI) N = 9	BoosterGMC (95% CI) N = 10	p-value
Vaccine serotypes						
1	1.893 (1.276–2.809)	1.42 (0.97–2.08)	0.161	2.636 (1.399–4.967)	2.599 (1.329–5.085)	0.448
5	0.643 (0.453–0.911)	0.898 (0.668–1.207)	0.100	1.091 (0.6–1.981)	1.026 (0.503–2.091)	0.716
6B	0.991 (0.654–1.502)	2.429 (1.655–3.563)	0.0003	0.56 (0.254–1.234)	1.416 (0.532–3.767)	0.0148
14	6.805 (4.277–10.827)	8.082 (6.123–10.668)	0.541	5.221 (3.025–9.01)	7.297 (4.474–11.901)	0.196
18C	3.455 (2.466–4.84)	2.368 (1.634–3.431)	0.012	8.024 (5.937–10.845)	7.954 (3.939–16.062)	0.4258
19F	2.908 (1.811–4.671)	4.385 (2.679–7.177)	0.098	4.581 (2.374–8.842)	10.196 (5.855–17.757)	0.004
23F	2.379 (1.368–4.139)	2.722 (1.783–4.157)	0.4123	0.491 (0.186–1.294)	1.065 (0.391–2.903)	0.039
Vaccine related serotypes						
6A	0.289 (0.169–0.496)	0.833 (0.459–1.513)	0.0005	0.218 (0.111–0.427)	0.583 (0.227–1.494)	0.006
19A	0.244 (0.177–0.335)	0.536 (0.284–1.013)	0.008	0.352 (0.158–0.787)	1.55 (0.634–3.789)	0.0004

(p = 0.06) among both groups for% of responders. The comparison of GMCs for the common seven serotypes shows significant differences for serotypes 18C (p = 0.001) and 19F (p = 0.031) between PCV7-TT and Synflorix®. It was detected significant differences in IgG GMC for serotype 19A (p = 0.046), but not for 6A serotype (p = 0.475) between both vaccines.

We analyzed the booster effects after complete 2p+1 schedule (Table 4), comparing the primary series (two doses) with booster dose for both vaccines. The GMCs increased significantly in vaccinated with PCV7-TT for serotypes 6B (p = 0.000), 18C (p = 0.0012), 6A (p = 0.0005) and 19A (0.008). In vaccinated with Synflorix® significant increasing was detected for serotypes 6B (p = 0.01), 19F (p = 0.004), 23F (p = 0.039), 6A (p = 0.006) and 19A (p = 0.0004). No booster effect for IgG GMC was detected for rest of serotypes in any group.

Table 5 shows the OPA responders percentage (subjects who achieved OPA antibody titers ≥1:8) and OPA GMTs in both vaccine groups. The proportion of responders to the seven common serotypes was ≥89.5% after the primary series and 100% after the booster dose in those vaccinated with PCV7-TT. In the Synflorix® vaccinated group, the percentage of responders to the seven common serotypes was ≥77.8% after the primary series and it exceeded 90% after the booster dose. The % of responders for related serotypes 6A and 19A was higher than 77% in both groups. OPA GMTs following the booster dose were significantly higher than the levels observed after the primary series for serotypes 1 (p = 0.001), 5 (p = 0.004), 6B (p = 0.03), 18C (p = 0.006) and 23F (p = 0.022) in the control group. In the PCV7-TT group, the OPA GMTs following the booster dose were significantly higher than primary doses for serotypes 1 (p = 0.0001), 5 (p = 0.004) and 6B (p = 0.003).

All subjects in both groups achieved protective titer levels against tetanus toxin (above 0.1 UI/ml). The IgG GMCs achieved in PCV7-TT vaccinated infants range from 5.5 UI/ml (CI 95% 4.17–7.7) after the primary series to 5.9 UI/ml (CI 95% 4.72–7.31) after the booster dose. In the Synflorix® vaccinated group this range was from 3.5 UI/ml (CI 95% CI 95% 2.058–5.965) after the primary series and 3.3 UI/ml (CI 95% 2.006–5.416) after the booster dose.

4. Discussion

We report the first results of the safety and immunogenicity of the Cuban-developed PCV7-TT among infants, compared to the widely used PCV-10 (Synflorix®). Among infants vaccinated with either vaccines, local reactions predominated and systemic events were mild. The new pneumococcal vaccine candidate PCV7-TT was immunogenic in the target infant population against the seven common serotypes included in the vaccine.

The trial was designed taking into account the schedule for the administration of pneumococcal vaccine comprising a two-dose primary series followed by a booster dose administrated at 11 months of age, as recommended by WHO [21]. Pneumococcal conjugate vaccines are regularly administrated in the first semester of life. However, because the carrier protein in PCV7-TT is tetanus toxoid, and this antigen is contained in other pediatric vaccine administrated in infant period, we conducted this clinical trial in the second semester of life (7, 8 and 11 months) in order to avoid interference with the immune response to these vaccines (for example the pentavalent vaccine against diphtheria, tetanus, pertussis, hepatitis B and *Haemophilus influenzae* type b). It doesn't mean this will be the final schedule for PCV7-TT.

The reactogenicity of PCV vaccines, including injection-site reactions and fever, has been evaluated in several randomized clinical trials and the findings of these trials are summarized in a systematic review [22].

The safety of the new Cuban pneumococcal vaccine had been previous explored in other age groups including adults [10] and preschool children [11]. The most frequently reported reactions were local, including tenderness, redness and swelling, and ranged from 26.7% to 53.25% in those vaccinated with Synflorix® and 27.8% to 58.2% in those vaccinated with PCV7-TT. The results were comparable to other clinical trials using Synflorix® [23,24] reporting redness at the injection site as the most frequent event after prime vaccination (55% of all doses) and pain at the injection site as the most common adverse events after the booster dose (51%). In the majority of cases, these adverse events were transient and mild to moderate in severity.

Our study did not identify any severe events associated with vaccination resulting in hospitalization, emergency or outpatient visits. The reported local and systemic reactions were typically mild and severe local reactions were uncommon and self-limited when they occurred. The consumption of antipyretic drugs occurred in approximately 40% of the vaccinated infants which is comparable to other studies using PCV vaccines [23].

The immunogenicity data in the current clinical trial is also consistent with previous studies of PCV10 administered in a simplified two-dose infant series followed by a booster [22–24]. In our study, both vaccines were immunogenic with two infant doses for the seven common pneumococcal serotypes. All subjects were responders to serotypes 1, 14, and 18C in both vaccine after primary series. For the remaining vaccine serotypes, more than 66.7% of infants achieved IgG concentration higher than 0.35 μg/ml. In the case of PCV7-TT, the percentage of responders after booster dose were 100% for all serotypes except serotype 5 (95%), while for Synflorix® it was 90% for serotypes 5 and 80% for serotype 23F. Similarly, other clinical trial using two dose of Synflorix have

Table 5
Pneumococcal OPA antibody titer ≥1:8 and pneumococcal OPA GMTs after primary series and booster dose.

Serotypes	PCV7-TT						Synflorix®					
	% responder OPA ≥ 1:8 (CI 95%)			GMT (CI 95%)			% responder OPA ≥ 1:8 (CI 95%)			GMT (CI 95%)		
	Infants series (N = 19)	Booster dose (N = 19)	p value	Primary series (N = 19)	Booster dose (N = 19)	p value	Primary series (N = 9)	Booster dose (N = 10)	p value	Primary series (N = 9)	Booster dose (N = 10)	p value
Vaccine serotypes												
1	89.5 (68.6, 97.1)	100 (83.2, 100)	0.486	38.4 (19.3, 76.5)	117.1 (59.9, 228.6)	0.0001	88.9 (56.5, 99.4)	90 (59.6, 99.5)	1	33.7 (12.7, 89.5)	112.88 (34.2, 372.5)	0.001
5	94.74 (75.4, 99.7)	100 (83.2, 100)	1	97.3 (56.4, 167.8)	226.1 (130.66, 391.3)	0.004	88.9 (56.5, 99.4)	100 (72.2, 100)	1	73.56 (22.2, 243.6)	313.48 (123.14, 798.0)	0.004
6B	94.74 (75.4, 99.7)	100 (83.2, 100)	1	2098.1 (864.9, 5089.3)	4410.3 (2641.68, 7362.9)	0.003	77.8 (45.3, 93.7)	90 (59.6, 99.5)	0.582	220.3 (36.6, 1325.9)	1224.7 (244.6, 6131.9)	0.03
14	100 (83.2, 100)	100 (83.2, 100)	–	13890.3 (9715.1, 19860)	13353.4 (9462.5, 18844.2)	0.679	100 (70.1, 100)	100 (72.2, 100)	–	1753.6 (1102.1, 2790.3)	2387.1 (1409.5, 4043.8)	0.142
18C	100 (83.2, 100)	100 (83.2, 100)	–	1871.3 (1256.0, 2787.8)	1873.7 (1208.2, 2905.7)	0.994	100 (70.1, 100)	100 (72.2, 100)	–	3165.26 (1952.4, 5131.5)	5888.7 (2853.7, 12151.7)	0.006
19F	94.74 (75.4, 99.7)	100 (83.2, 100)	1	1323.4 (589.7, 2970.2)	1799.0 (1171.7, 2762.2)	0.922	100 (70.1, 100)	100 (72.2, 100)	–	1748.22 (1008.7, 3030)	2693.68 (1501.7, 4831.6)	0.074
23F	100 (83.2, 100)	100 (83.2, 100)	–	6123.3 (3869.1, 9691)	8150.6 (4934.10, 13464.1)	0.324	87.5 (52.9, 99.4)	90 (59.6, 99.5)		446.1 (76.9, 2588.8)	1095.3 (225, 5332.6)	0.022
Vaccine related serotypes												
6A	78.95 (56.67, 91.49)	94.74 (75.36, 99.73)	0.340	1119.8 (245.9, 5099.3)	2205.9 (820.4, 5931.1)	0.514	62.5 (30.57, 86.32)	77.78 (45.26, 93.68)	0.620	102.8 (10.4, 1017.6)	360.9 (44.3, 2941.9)	0.059
19A	100 (82.41, 100)	78.95 (56.67, 91.49)	0.105	80.8 (43.7, 149.1)	170.8 (59.7, 488.2)	0.002	88.89 (56.5, 99.43)	100 (72.25, 100)	0.474	130.13 (35.1, 483.1)	817.8 (278.2, 2404.4)	0.003

reported lower percentage of responders for serotype 6B and 19F post primary series [17].

Opsonophagocytic antibodies induced for the new pneumococcal vaccine is one of the main finding of this study. Despite the variation between vaccine serotypes, more than 89% of subject vaccinated with PCV7-TT achieving OPA titer above 1:8 after primary series and 100% after booster dose respectively. An interest although preliminary result was the immune response of PCV7-TT to additional serotypes 6A and 19A. These serotypes are not included in the vaccine candidate, however, after booster dose 94.7% and 78.95% of subjects vaccinated with PCV7-TT were responders (OPA ≥ 1:8) for 6A and 19A respectively. National Reference Laboratory Surveillance [25] and NP colonization studies [7,26] done in Cuba have identified serotype 19A as relevant, then it will be very important to study deeply the possible crossprotection between 19F and 19A in PCV7-TT vaccinated infants through non-inferiority immunogenicity studies.

There is no other licensed PCV using only tetanus toxoid as carrier protein. Then, in one way the possible interference with other pediatric vaccines should be deeply studied, but on other side, the possible priming role of tetanus toxoid could be observed, as reported in studies with *Haemophilus* conjugated vaccines [27]. This hypothesis will be tested in Phase I/II study started in Cuba in 2017 exploring the immunogenicity in a cohort of approximately 880 infants 2–3-months old using different vaccination schedules (2p+1 and 3p+0).

The main limitation of the current study is the lower number of enrolled infants that limited the probability of detecting a significant difference among the vaccination groups in terms of immunogenicity. However, these results support further steps of clinical evaluation in this target group since the new vaccine is well tolerated in infants.

5. Conclusions

Our results showing an acceptable safety profile and immunological results for PCV7-TT administered in a two-dose primary series and a booster dose (2p+1) schedule in healthy infants vaccine-naïve for pneumococcal vaccination. It supports further studies included in clinical evaluation strategy of PCV7-TT.

The immune response generated by PCV7-TT suggests that it could provide protection against pneumococcal diseases. The comparable immunogenicity and safety profiles of PCV7-TT and Synflorix® suggest PCV7-TT could have a valuable addition to the national immunization schedule in Cuba and in other resource-limited settings.

Declaration of interests

Main authors of this paper: Nivaldo Linares-Pérez, PhD; Beatriz Paredes, BSc; Mayelín Mirabal, MSc; Laura M. Rodriguez-Noda, BsC; Darielys Santana, BsC; Dagmar García-Rivera, PhD; Yury Valdés Balbín, BSc and Vicente Verez-Bencomo, PhD, work at the manufacturing vaccine center "Finlay Vaccine Institute". The rest of main authors: María E. Toledo-Romaní, PhD; Carlos P. Dotres, MD; Rinaldo Puga, MD, Yariset Ricardo, MD; working in the National Health System, and neither had contracts or received financing from the manufacturing center.

Contributions of authors

Nivaldo Linares-Pérez, PhD, María E. Toledo-Romaní, PhD and Mayelín Mirabal, MSc, analyzed and interpreted the data and designed and wrote this paper. Carlos P. Dotres, MD; Beatriz Paredes, BSc; Rinaldo Puga, MD; Dagmar García-Rivera, PhD; Yariset

Ricardo, MD; Carmen R. Broño, MD, participated the conception and design of the study and acquisition of data of this clinical trial. Laura M. Rodríguez, BsC; Dagmar García-Rivera, PhD; and David Goldblatt were in charge of the processing and evaluation of laboratory samples. All authors were involved as experts in the discussion, review and final approval of the version to be submitted.

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