Vaccine 37 (2019) 3840–3848

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Evolution of serotypes in bacteremic pneumococcal adult pneumonia in the period 2001–2014, after introduction of the pneumococcal conjugate vaccine in bizkaia (spain)

Pedro P. España [a,*], Ane Uranga [a], Luis Alberto Ruiz [b], Jose María Quintana [c], Amaia Bilbao [d], Amaia Aramburu [a], Leyre Serrano [b], Rafael Ayarza [e], Ana Patricia Martinez [e], Rafael Zalacain [b]

[a] Department of Respiratory Medicine, Galdakao-Usansolo Hospital, Bizkaia, Spain
[b] Department of Respiratory Medicine, Cruces University Hospital, Bizkaia, Spain
[c] Research Unit, Galdakao-Usansolo Hospital – Health Services Research on Chronic Patients Network (REDISSEC), Bizkaia, Spain
[d] Research Unit, Basurto Hospital – Health Services Research on Chronic Patients Network (REDISSEC), Bizkaia, Spain
[e] Department of Microbiology, Galdakao-Usansolo Hospital, Bizkaia, Spain

ARTICLE INFO

Article history:
Received 22 December 2017
Received in revised form 13 May 2019
Accepted 16 May 2019
Available online 29 May 2019

Keywords:
Bacteremic pneumococcal pneumonia
Pneumococcal conjugate vaccine
Pneumococcal serotypes
Comorbidities
Clinical outcomes

ABSTRACT

The introduction of pneumococcal conjugate vaccines (PCV7 and PCV13) in children has led to a change in the pattern of pneumococcal serotypes causing pneumococcal disease in adults. The aim of this study is to analyze the distribution of pneumococcal serotypes in adults with bacteremic pneumococcal community-acquired pneumonia (BPP) after the introduction of PCVs in childhood, and the impact of age and comorbidity on this distribution. We conducted an observational study of all adults hospitalized with BPP between 2001 and 2014, in two tertiary hospitals. Overall, we identified 451 cases of BPP (2001–2005: 194, 2006–2010: 134, 2011–2014: 123). The rate of appearance of new cases decreased over the study period. In 70% of the cases, the serotypes found were among those included in PCV13. The most prevalent serotypes were 3 (23.1%), 7F (14.6%), 19A (8.4%) and 1 (7.5%). There was a significant trend to decrease in the percentage of BPP cases due to PCV7 from period 2001–2005 to 2011–2014 ($p = 0.0166$) and a significant trend to increase in the six serotypes added to form PCV 13 ($p = 0.0003$). Serotype 3 was the most frequent in patients who developed complications during hospitalization. We did not detect a significant increase in cases caused by non-PCV13 serotypes. The most frequent non-PCV13 serotype was 22F. In conclusion, a significant proportion of adults continue to develop BPP with vaccine serotypes despite infant pneumococcal vaccination. There is a need for further strategies to reduce the current burden of this disease on adults.

1. Introduction

Pneumococcal infection is a major public health problem worldwide because of its high morbidity and mortality rates [1–3]. *Streptococcus pneumoniae* is the most common cause of pneumonia, being found in 10–15% of cases with bacteremia, and traditionally associated with poor outcomes [4,5].

Immunological protection against pneumococcal pneumonia is mediated through antibodies against bacterial capsular polysaccharides that define the pneumococcal serotypes and serve as virulence factors [6]. For that reason, a number of vaccines have been developed seeking to cover the most prevalent serotypes. Currently, there are two types of pneumococcal vaccines, both directed against a limited number of these serotypes: the 23-valent pneumococcal polysaccharide vaccine (PPSV23) and the protein-conjugated vaccines directed against 7 (PCV7), 10 (PCV10) or 13 (PCV13) serotypes. For prevention in children <2 years, only conjugated vaccines (PCVs) have been approved, whereas for the adult population both have: first, the polysaccharide vaccine (PPSV23), and more recently PCV13, approved for having demonstrated greater effectiveness in the prevention of pneumococcal pneumonia.

In Spain, childhood pneumococcal conjugate vaccination began in 2001 with PCV7, while the newer PCV13 became available in 2010. During the study period (2001–2014), these vaccines were not being covered by the Spanish National Health Service, but could be administered if bought privately. In addition coverage

* Corresponding author.
E-mail address: pedropablo.espanayandiola@osakidetza.eus (P.P. España).

https://doi.org/10.1016/j.vaccine.2019.05.052
0264-410X/

varied between regions of the country. The uptake in the area where the study was carried out was estimated to be about 61% for the period 2010–2014 [7].

However, recently available data suggest that PCV13 pneumococcal pneumonia serotypes continue to cause a high rate of infection in adults, despite some impact of childhood PCV13 vaccination [8]. Further, given that PCV13 has recently been evaluated and approved for the prevention of pneumonia in adult patients [9], it is essential to determine the distribution of serotypes in adult BPP to estimate and then assess the impact of implementation of standard vaccination in adults.

The primary objective of the present study is to investigate the distribution of the pneumococcal serotypes included in PCV13 in adults with BPP over time, since the introduction of PCV7 and subsequently of PCV13 vaccination in children. Secondary objectives were to evaluate serotype replacement by non-PCV13 serotypes and the impact of age and comorbidities on the serotype distribution.

2. Methods

2.1. Patients and study design

This was a prospective observational study of consecutive patients (≥18 years of age) hospitalized for BPP in two tertiary hospitals (Cruces and Galdakao-Usansolo) in the Basque Country (Spain), with a catchment population of around 700,000. The study was conducted between January 2001 and December 2014. The ethics committees of both hospitals approved the study.

For a patient to be classified as having BPP, we required the presence of new pulmonary infiltrate on chest X-ray together with acute signs and symptoms suggestive of lower respiratory tract infection in addition to one positive blood culture for *S. pneumoniae* taken within 24 h of presentation at hospital. Individuals were excluded if they were known to be positive for human immunodeficiency virus or chronically immunosuppressed, as well as if they had had an episode of pneumonia in the 3 months prior or if they had hospital-acquired pneumonia.

Patients were considered active smokers when they smoked at least 10 cigarettes per day and heavy alcohol users if they reported a daily alcohol intake of at least 80 g for men or 60 g for women during the previous year [10]. Vaccine status refers to having received the influenza vaccine the previous year and the polysaccharide vaccination PPSV23 in the previous 5 years. No patient had been vaccinated with the PCV13 conjugate during the study period. Septic shock was defined as systolic blood pressure <90 mm Hg and the need for a vasopressor agent for at least 4 h, after fluid therapy.

Patients were treated empirically at the discretion of the attending doctor following the guidelines of the Spanish Society of Pulmonology and Thoracic Surgery [11].

2.2. Study variables

Data were collected on patient clinical and demographic characteristics, vaccination status, antibiotic treatment during the previous month, blood test results and X-ray findings. To assess the severity of a patient's pneumonia on admission, we used the Pneumonia Severity Index [12]. Two consecutive blood cultures were performed for all patients within 24 h after hospital admission.

The course of the illness and patient outcomes were described using the following variables: admission to intensive care unit (ICU); use of invasive mechanical ventilation; development of septic shock; 30-day mortality; and development of pleural effusion.

The study was divided into three periods (first, 2001–2005 vs. second, 2006–2010, vs. third period, 2011–2014).Serotypes were categorized into three groups: PCV 7 serotypes, the six serotypes added in PCV13 (PCV13-7), and the non-vaccine serotypes (non-PCV13)

Pneumococcal isolates were stored frozen in skimmed milk at −40 °C or below in the microbiology department at each hospital until delivery to the central laboratory (Reference Laboratory of the Carlos III Health Institute). Serotyping was performed by multiplex polymerase chain reaction [13] and confirmed by the Quellung reaction using polyclonal antisera.

2.3. Statistical analysis

Patients with BPP whose serotype was not determined were described as having "untyped pneumococcal CAP". We compared the main characteristics between patients with and without serotyping data. Among serotyped patients, we compared the main characteristics between the two participating hospitals. For categorical variables, chi-square or Fisher's exact tests were used for comparisons, and for continuous variables t-tests or the nonparametric Wilcoxon tests.

Patient general characteristics, comorbidities, and outcomes were compared among the three study periods. The serotype distribution was compared between the three periods in the entire study population and in the following subsamples: patients with comorbidities, patients that were otherwise healthy, patients with risk factors, and patients by age group (<50 years, 50–64 years, or ≥65 years). We used chi-square or Fisher's exact tests for the comparison of categorical variables and analysis of variance or nonparametric Kruskal-Wallis tests for continuous variables. The Bonferroni correction was used for multiple comparisons. Further, the Cochran-Armitage trend test was also used to study trends among the three periods. To draw inferences about changes in the period before PCV 13 introduction in children on the period after, serotype distribution during 2001–2010 was compared with distribution during 2011–2014, by means of the chi-square or Fisher's exact test.

Further, we compared outcomes by serotype. Each outcome of each serotype was compared with respect to serotype 1. We used serotype 1 as the reference group because it represents a frequent serotype in our study, and had a high risk of invasive disease and low fatality rate [14]. We used chi-square or Fisher's exact tests with Bonferroni correction for multiple comparisons. In addition, we analyzed the relationship between serotypes and outcomes adjusting for other covariates, such as age, sex, comorbidities and pneumococcal vaccination, by means of multivariate logistic regression models.

All effects were considered statistically significant at $p < 0.05$. All statistical analyses were performed using SAS for Windows, version 9.2 (SAS Institute Inc., Cary, NC).

3. Results

Out of 529 patients with BPP evaluated, no serotypes were collected (untyped pneumococcal) in 78 of them, so they were excluded from the study, resulting therefore in a study cohort of 451 patients. There were no significant differences in age ($p = 0.8982$) or in comorbidities ($p = 0.8387$) between serotyped and non-serotyped patients.

Overall, there was a decrease in the frequency of new cases of BPP over the study period. There were no significant differences between the two hospitals in the presence of comorbidities ($p = 0.5703$), severity ($p = 0.1844$), 30-day mortality ($p = 0.7716$) and serotype distribution ($p > 0.5854$). There were significant dif-

ferences in age (p = 0.0003), mechanical ventilation (p = 0.0265) and ICU admission (p = 0.0201) (Table 1S supplement on line).

3.1. Characteristics, comorbidities and outcomes

Patient sociodemographic characteristics, comorbidities and outcomes during the three study periods are summarized in Table 1. Considering the entire sample, 53.9% of patients were ≥65 years old and 46.7% had some comorbidity. The most common comorbidity was chronic obstructive pulmonary disease (COPD) (17.2%), followed by diabetes (15.1%) and heart failure (12.9%). Further, 31.2% were active smokers and 12% reported heavy alcohol user. A third (33%; 151/451) of patients with BPP had no comorbidities, and reported neither active smoking nor heavy alcohol use at baseline.

Overall, 30-day mortality was 7.8% and a significant decrease was observed during the study period (from 11.3% during the first period [2001–2005] to 2.5% in the third period [2011–2014], trend test p = 0.0041). Mortality was higher in patients ≥65 years of age (9.1%) and in those with comorbidities (10.5%). Within these subgroups we also observed a significant decrease in mortality. In the group of patients ≥65 years, mortality decreased significantly from 12% in the period 2001–2005 to 1.3% in the period 2011–2014 (trend test p = 0.0348). A similar trend was observed in the group with comorbidities, with a decrease from 13.1% in the first period to 3.7% in the last, although it didn't reach statistical significance (trend test p = 0.0850)

The overall rates of complications were as follows: 25.3% of patients needed ICU admission, 14.8% developed septic shock, 10.6% needed mechanical ventilation and 16% developed metapneumonic effusion. Table 2 details the distribution of complications across the serotypes. Serotype 1 was not responsible for any deaths, but was the most closely associated with pleural effusion (Table 2). We found statistically significant differences in septic shock and mechanical ventilation of serotype 3 (30.2% and 25%, respectively) in relation to serotype 1 (0% and 2.9%, respectively). The percentage of septic shock was also significantly higher in patients with serotype 22F when compared with serotype 1 (25%

Table 1
General characteristics of the study sample.

Variable	2001–2005 [a] (N = 194)	2006–2010 [a] (N = 134)	2011–2014 [a] (N = 123)	p
Male	135 (69.6)	85 (63.4)	79 (64.2)	0.434
Age (years), mean (SD)	65 (17.2)	60.8 (20.2)	62.3 (17.5)	0.108
Nursing home resident	7 (3.6)	6 (4.5)	1 (0.8)	0.207
Smoker	32 (28.8)	38 (29.5)	43 (35.3)	0.496
Alcohol user	24 (12.8)	18 (14.5)	10 (8.3)	0.295
Pneumococcal vaccine (PPSV23)	2 (1.2)[a]	7 (6.1)	11 (13.3)[a]	0.0003
Influenza vaccine	35 (19.8)[a]	44 (37.9)[a]	20 (24.1)	0.002
Comorbidities				0.008
0	86 (44.6)[a]	85 (63.9)[a]	68 (55.7)	
1	72 (37.3)[a]	32 (24.1)[a]	31 (25.4)	
>1	35 (18.1)	16 (12)	23 (18.9)	
Pneumonia Severity Index class IV-V	106 (54.6)	66 (49.3)	61 (49.6)	0.546
Appropriate antibiotic (SEPAR)	112 (57.7)[a]	116 (86.6)[a]	96 (80.0)[a]	<0.0001
Length of stay (days)				
Mean (SD)	9.1 (11.5)	10.2 (17.5)	9.4 (16.7)	0.343
Median (IQR)	6 (4–9)	5 (3–9)	5 (4–10)	0.343
30-day mortality	22 (11.3)[a]	10 (7.5)	3 (2.5)[a]	0.016
Septic shock	25 (14.7)	20 (16.3)	22 (17.9)	0.764
Mechanical ventilation	23 (11.9)	15 (11.2)	10 (8.1)	0.560
Pleural effusion	26 (13.4)	29 (21.6)	17 (13.8)	0.101
Intensive care unit admission	43 (22.2)	34 (25.6)	37 (30.1)	0.287
30-day readmission	3 (2.3)	4 (3.7)	2 (1.7)	0.606

SD: standard deviation; IQR: interquartile range.
Cochran-Armitage trend test resulted statistically significant for Pneumococcal vaccine (PPSV23) (p < 0.0001), Appropriate antibiotic (SEPAR) (p < 0.0001), and 30-day mortality (p = 0.0041).
[a] Superscript letters indicated significant differences among periods using Bonferroni correction for multiple comparisons, considering a p-value significant at p < 0.0166.

Table 2
Serotype distribution in relation to clinical outcomes.

Serotypes	Total number of cases	30-day mortality	Intensive care unit admission	Septic shock	Mechanical ventilation	Pleural effusion
N (%)	451	35 (7.8)	114 (25.3)	67 (14.8)	48 (10.6)	72 (16)
1 (reference)	34 (7.5)	0 (0)	6 (17.7)	0 (0)	1 (2.9)	10 (29.4)
3	104 (23.1)	14 (13.5)	43 (41.4)	29 (30.2)*	26 (25)*	18 (17.3)
7F	66 (14.6)	1 (1.5)	15 (22.7)	8 (13.6)	4 (6.1)	11 (16.7)
19A	38 (8.4)	2 (5.3)	8 (21.1)	4 (10.8)	3 (7.9)	9 (23.7)
14	33 (7.3)	4 (12.1)	3 (9.1)	3 (9.7)	2 (6.1)	5 (15.2)
4	33 (7.3)	1 (3)	9 (27.3)	6 (18.8)	3 (9.1)	8 (24.2)
8	32 (7.1)	3 (9.4)	7 (21.9)	4 (15.4)	2 (6.3)	5 (15.6)
22F	20 (4.4)	3 (15)	5 (25)	5 (25)*	3 (15)	0 (0)
9 V	10 (2.2)	1 (10)	0 (0)	0 (0)	0 (0)	1 (10)
Others	81 (18)	6 (7.4)	18 (22.5)	6 (11)	4 (4.9)	5 (6.2)*

PCV7: serotypes 4, 6B, 9 V, 14, 18C, 19F, and 23F; PCV13-7: serotypes included in PCV13 but not PCV7, i.e., 1, 3, 5, 6A, 7F, and 19A; PCV13: serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9 V, 14, 18C, 19A, 19F, and 23F; Non-PCV13: other serotypes not included in PCV13.
* Statistically significant differences with serotype 1 are indicated. The chi-square test or Fisher's exact test comparing clinical outcomes in each serotype in relation to serotype 1. The Bonferroni correction for multiple comparison was applied, considering a p-value significant at p < 0.0056.

vs. 0%, respectively). These differences remained statistically significant in the multivariate analyses after adjusting for age, sex, comorbidities and pneumococcal vaccine status. Besides, after adjustments, we also found significant differences in intensive care unit admission in serotype 3 in relation to serotype 1 (OR = 5.3; 95% confidence interval = 1.8–15.8; p = 0.0030) .

3.2. Distribution of serotypes

The distribution of serotypes was similar in the two hospitals (p > 0.5854) in the study periods (Table1S supplement, on line). Overall, of 451 BPP cases 318 (70.5%) were caused due to PCV13 serotypes.

Fig. 1 shows a significant decrease in the percentage of cases of the serotypes included in the initial PCV7 vaccine from period 1–3 (trend test p = 0.0166), and a significant increase in the six serotypes that were added to form PCV13 (trend test p = 0.0003). More specifically, regarding PCV13-7, there was a significant difference (p = 0.0011) between the first period (41.8%) and the second period (61.8%) but not between the second and third periods (56% and 61.8% respectively). A non-significant decrease was observed in non-PCV 13 serotypes. The most prevalent serotypes were 3 (23.1%), 7F (14.6%), 19A (8.4%), and 1 (7.5%).

After the introduction of the PCV13 vaccine in 2010, among the six extra serotypes (PCV13-7), serotypes 1 and 5 showed a non-significant decrease over this period, serotypes 3, and 19A showed a non significant increase, while the presence of the 7F serotype was the only one that increased significantly (Fig. 2).

With respect to serotype replacement, we did not identify a significant increase in cases caused by non-PCV13 serotypes. Among the most frequent non-PCV13 serotypes, serotype 22F showed a non-significant increase since the introduction of the PCV13 vaccine, while serotype 8 decreased in 2006–2010, but remained stable in the following period (2011–2014)), with a significant trend (p = 0.0146) (Fig. 3).

Figs. 4a and b shows the distribution of serotypes by health status and age. The most prevalent serotypes in patients with comorbidities and those ≥65 years of age were included in PCV13, accounting for as many as 71.2% of cases (173/243). Significant changes were observed in the distribution of serotypes in the two subgroups: in patients with comorbidities for PCV7 (trend test p = 0.0010) and PCV13-7 (trend test p = 0.0011) and in those ≥65 years of age, with a significant decrease for PCV 7 (trend test p = 0.0052), and with a significant increase in the six serotypes that were added in PCV13 (trend test p = 0.0006).

4. Discussion

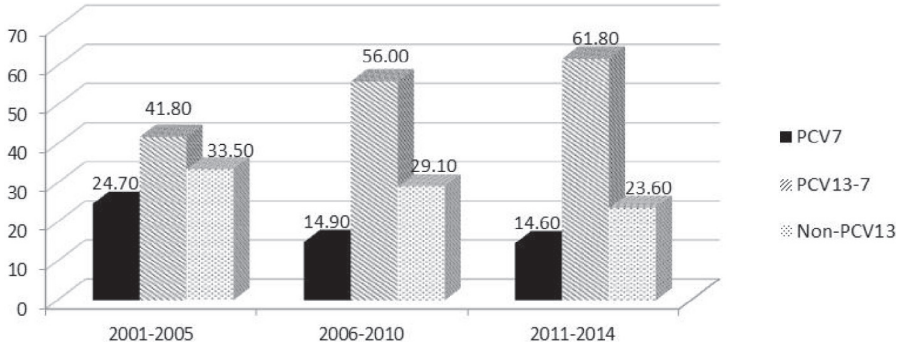
The most notable findings of the study were: (i) the serotypes included in PCV13 were responsible for 70.5% of BPP in adults; (ii) there was a decrease trend for the serotypes included in PCV7 and a increasing trend in some of the six additional serotypes in PCV13; (iii) the most prevalent serotypes were: 3, 7F, 19A and 1; and (iv) in the last 4 years, even after the introduction of the PCV13 infant vaccine, the most prevalent serotypes in BPP in adults were still found among those included in PCV13, with no replacement effect seen by non-PCV13 serotypes.

4.1. PCV7 serotype decrease vs PCV13 serotype increase

Following the effect found after the implementation of the PCV7 vaccine in 2001 in both the USA and Europe [15], a parallel decrease in the six additional serotypes included in PCV13 would be expected after the introduction of the PCV13 vaccine in 2010. The reduction in these six serotypes, has indeed been observed in countries with high immunization coverage, such as England, Wales and the USA (immunization rates above 90%) [16,17].

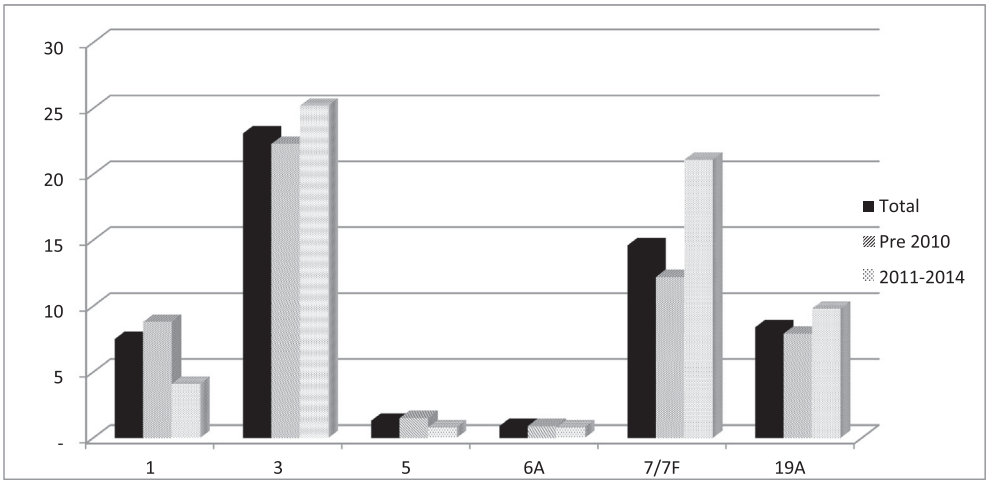
Our data do not follow this trend. They suggest that the indirect effect of childhood immunization programs, when vaccination coverage is lower than in the aforementioned countries, may have reduced disease among adults due to PCV7 serotypes but not eliminated them. While we saw an increase in BPP due to additional serotypes in PCV13 from 2001 to 2005 period to 2006–2010, there was no decline in the post-PCV13 2011–2014 period.

Further, the herd protection effects post-PCV13 seem to be less pronounced than the equivalent effects post-PCV7. This pattern has also been found in the work of Galanis et al. [18] in an elderly population. In an Italian study, conducted in a large population of



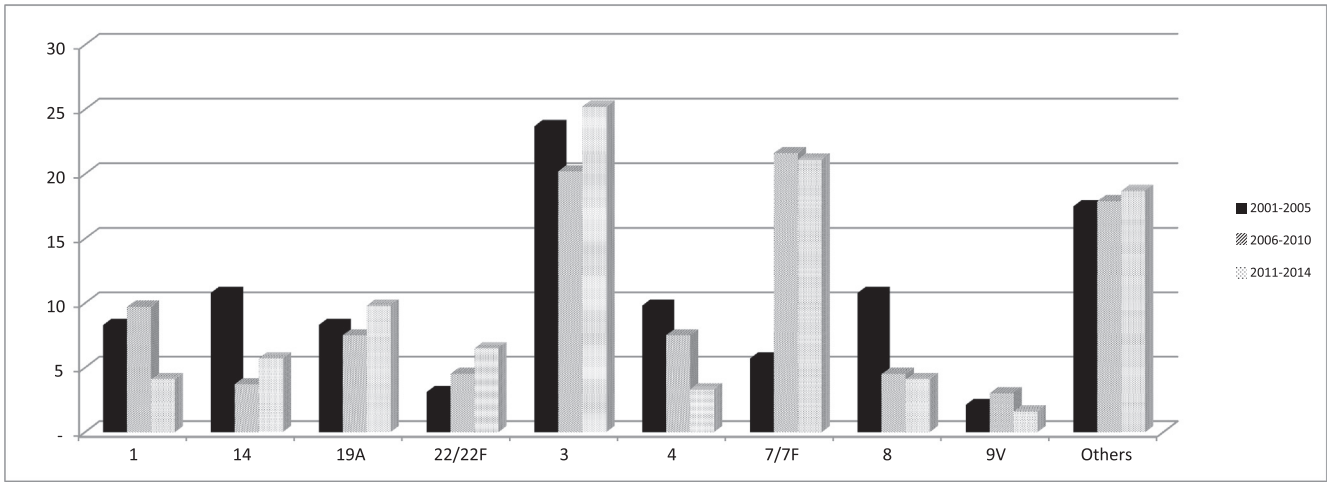
	2001-2005 [a]	2006-2010 [b]	2011-2014 [c]	p value
	n (%)	n (%)	n (%)	
PCV7	48 (24.7)	20 (14.9)	18 (14.6)	0.0287
PCV13-7	81 (41.8)[b,c]	75 (56)[a]	76 (61.8)[a]	0.0011
Non-PCV13	65 (33.5)	39 (29.1)	29 (23.6)	0.1668

Fig. 1. Evolution of vaccine (PCV7 and PCV13-7) and non-vaccine (non-PCV13) serotypes by time period. PCV7: serotypes 4, 6B, 9 V, 14, 18C, 19F, and 23F; PCV13-7: serotypes 1, 3, 5, 6A, 7F, and 19A; Non-PCV13: other serotypes, not included in PCV13. The p values measures the homogeneity of the distribution of serotypes between the three periods of time by means of the Chi-square test. [abc] Superscript letters indicated significant differences between the periods using Bonferroni correction for multiple comparisons, considering a p-value significant at p < 0.0166. Cochran-Armitage trend test resulted statistically significant for PCV7 (p = 0.0166) and PCV13-7 (p = 0.0003), but not for Non-PCV13 (p = 0.0589).



	1	3	5	6A	7/7F	19A
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
Total	34 (7.5)	104 (23.1)	6 (1.3)	4 (0.9)	66 (14.6)	38 (8.4)
Pre 2010	29 (8.8)	73 (22.3)	5 (1.5)	3 (0.9)	40 (12.2)	26 (7.9)
2010-2014	5 (4.1)	31 (25.2)	1 (0.8)	1 (0.8)	26 (21.1)	12 (9.8)
p value	0.0871	0.5081	1	1	0.0167	0.5330

Fig. 2. Evolution of the six extra serotypes added to PCV13, overall and before and after introduction of PCV13. The p values measures the homogeneity of the distribution of serotypes between the two periods of time by means of the Chi-square test or Fisher's exact test.



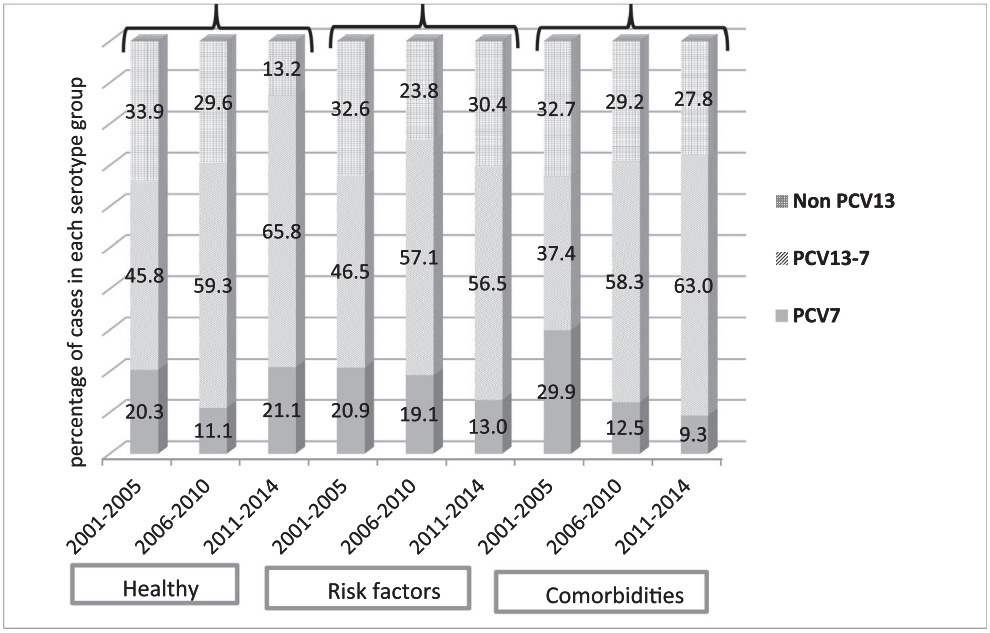
	n	1	14	19A	22/22F	3	4	7/7F	8	9V	Others
		n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
2001-2005[a]	194	16 (8.3)	21 (10.8)	16 (8.3)	6 (3.1)	46 (23.7)	19 (9.8)	11 (5.7)[b,c]	21 (10.8)	4 (2.1)	34 (17.5)
2006-2010[b]	134	13 (9.7)	5 (3.7)	10 (7.5)	6 (4.5)	27 (20.2)	10 (7.5)	29 (21.6)[a]	6 (4.5)	4 (3)	24 (17.9)
2011-2014[c]	123	5 (4.1)	7 (5.7)	12 (9.8)	8 (6.5)	31 (25.2)	4 (3.3)	26 (21.1)[a]	5 (4.1)	2 (1.6)	23 (18.7)
p value		0.2051	0.038	0.798	0.3556	0.6051	0.0927	<0.0001	0.0273	0.7467	0.9653

Fig. 3. Changes in the number of cases of the most prevalent serotypes over the study period. The p values measures the homogeneity of the distribution of serotypes between the three periods of time by means of the Chi-square test. [abc] Superscript letters indicated significant differences between the periods using Bonferroni correction for multiple comparisons, considering a p-value significant at $p < 0.0166$. Cochran-Armitage trend test resulted statistically significant for serotypes 4 ($p = 0.0313$), 7/7F ($p < 0.0001$) and 8 ($p = 0.0146$).

adults where there was high coverage of infant pneumococcal vaccination, it was also found that serotypes 1, 3, 7F and 19A caused most cases of BPP in adults [19]. So far, it remains questionable whether herd protection effects apply uniformly to all serotypes beyond PCV7, as has been described for serotype 3 [16,17].

4.2. Frequency of serotypes

The most frequent serotypes found in Europe are, 1, 3, 7F, 14 and 19A [20]. Our results corroborate these data, with 3, 7F, 19A, 1 and 14 (all included in PCV13) being the most frequent. In Spain,



Group	Serotype	2001-2005 [a]	2006-2010 [b]	2011-2014 [c]	p value
		n (%)	n (%)	n (%)	
Healthy adults (N = 151)	PCV7	12 (20.3)	6 (11.1)	8 (21.1)	0.3315
	PCV13-7	27 (45.8)	32 (59.3)	25 (65.8)	0.1222
	Non-PCV13	20 (33.9)	16 (29.6)	5 (13.2)	0.0711
Adults with risk factors (N = 131)	PCV7	9 (20.9)	8 (19.1)	6 (13)	0.5916
	PCV13-7	20 (46.5)	24 (57.1)	26 (56.5)	0.5389
	Non-PCV13	14 (32.6)	10 (23.8)	14 (30.4)	0.6506
Adults with comorbidities (N =209)	PCV7	32 (29.9)[c]	6 (12.5)	5 (9.3)[a]	0.0027
	PCV13-7	40 (37.4)[b,c]	28 (58.3)[a]	34 (63)[a]	0.0029
	Non-PCV13	35 (32.7)	14 (29.2)	15 (27.8)	0.7893

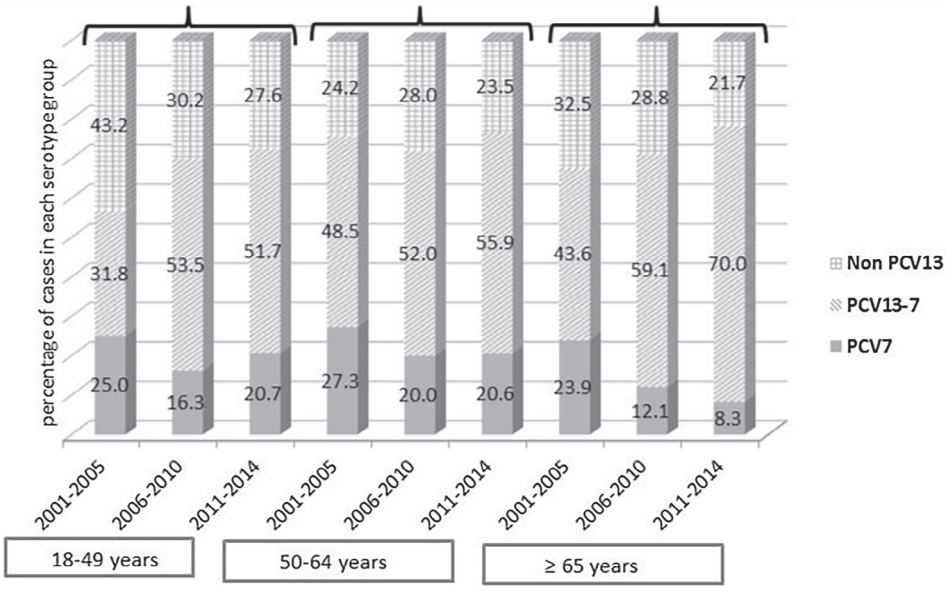
Fig. 4a. Evolution of serotypes in each period by subgroup: patients who were otherwise healthy, those with risk factors and those with comorbidities. PCV7: serotypes 4, 6B, 9 V, 14, 18C, 19F, and 23F; PCV13-7: serotypes 1, 3, 5, 6A, 7F, and 19A; Non-PCV13: other serotypes, not included in PCV13. Risk factor: Smoker and alcohol user; Comorbidities: chronic obstructive pulmonary disease, diabetes, heart failure, chronic liver disease, cancer, renal failure, or stroke. The p values measures the homogeneity of the distribution of serotypes between the three periods of time by means of the Chi-square test or Fisher's exact test. [abc] Superscript letters indicated significant differences between the periods using Bonferroni correction for multiple comparisons, considering a p-value significant at $p < 0.0166$. Cochran-Armitage trend test resulted statistically significant for PCV13-7 ($p = 0.0445$) and non-PCV13 ($p = 0.0311$) in healthy adults; and for PCV7 ($p = 0.0010$) and PCV13-7 ($p = 0.0011$) among adults with comorbidities.

data obtained from the multicenter ODIN study indicate that PCV13 serotypes were responsible for 63% of the invasive forms of disease in immunocompetent adults and 45% in immunocompromised patients [21]. The most frequently identified serotypes were 3 (11.2%), 19A (8.9%) and 7F (8.3%). In addition, the work of Ardanuy et al. [22] on episodes of invasive pneumococcal disease with patients ≥65 years found that PCV13 serotypes (19 A, 3, 7F, 14, 1) were the most prevalent, causing 59.3% of episodes.

Our distribution of serotypes did not vary significantly in subgroups of patients with associated risk factors, comorbidity, or advanced age, even though increased age and comorbidity are recognized risk factors for invasive pneumococcal disease and these at-risk groups are targets for disease prevention [23,24]. Recently, a document endorsed by 18 scientific societies was published in Spain, with recommendations on pneumococcal vaccination in adults with underlying pathologies and conditions, among which the age-related recommendations stood out [25]. In contrast, one interesting finding in our study is that in patients with no underlying conditions, the proportion of BPP due to PCV13 serotypes was 72.2% overall and 86.8% in the post-PCV13 period 2010–2014. The potential of preventing cases of pneumonia among healthy adults should also be considered by vaccine policy makers when discussing adult PCV13 recommendations.

Serotype 3 was the most frequent and the one most associated with complications during hospitalization. Among all BPP cases serotype 1 had caused no deaths and was the serotype most associated with metapneumonic pleural effusion. These patterns have also been observed in previous studies [26,27]. Considering the variables for which we had data, the observed decrease in mortality was related to an increase in the use of the appropriate antibiotics, vaccination with PPSV23 among adults with BPP, and the conjugated vaccination in children during the period of study [28].

In our study area, current data suggest that serotypes 3, 7F and 19A have not been affected by the administration of PCV13 in childhood and remain the most common serotypes found among adults with BPP. As a comparison, in the CAPITA study [29], a double blind, placebo-controlled trial in patients over 65 years old, in which participants were randomized to receive either a single dose of PCV13 or a placebo, it was demonstrated that the highest efficacy for PCV13 was found against the following three serotypes: 3, 7 and 19A [30].



Age group	Serotype	2001-2005 [a]	2006-2010 [b]	2011-2014 [c]	p value
		n (%)	n (%)	n (%)	
18-49 years (N = 116)	PCV7	11 (25)	7 (16.3)	6 (20.7)	0.6041
	PCV13-7	14 (31.8)	23 (53.5)	15 (51.7)	0.0875
	Non-PCV13	19 (43.2)	13 (30.2)	8 (27.6)	0.2970
50-64 years (N = 92)	PCV7	9 (27.3)	5 (20)	7 (20.6)	0.7481
	PCV13-7	16 (48.5)	13 (52)	19 (55.9)	0.8321
	Non-PCV13	8 (24.2)	7 (28)	8 (23.5)	0.9188
≥ 65 years (N =209)	PCV7	28 (23.9)[c]	8 (12.1)	5 (8.3)[a]	0.0155
	PCV13-7	51 (43.6)[c]	39 (59.1)	42 (70)[a]	0.0025
	Non-PCV13	38 (32.5)	19 (28.8)	13 (21.7)	0.3229

Fig. 4b. Distribution of serotypes in each period by age group. PCV7: serotypes 4, 6B, 9 V, 14, 18C, 19F, and 23F; PCV13-7: serotypes 1, 3, 5, 6A, 7F, and 19A; Non-PCV13: other serotypes, not included in PCV13. The p values measures the homogeneity of the distribution of serotypes between the three periods of time by means of the Chi-square test or Fisher's exact test. [abc] Superscript letters indicated significant differences between the periods using Bonferroni correction for multiple comparisons, considering a p-value significant at $p < 0.0166$. Cochran-Armitage trend test resulted statistically significant for PCV7 ($p = 0.0052$) and PCV13-7 ($p = 0.0006$) among patients ≥65 years.

4.3. Lack of replacement effect

We do not yet know the scale of the serotype replacement phenomenon in our area after the introduction of PCV13 vaccination in infants. In a neighboring region, after the introduction of PCV13, no increase in non-vaccine serotypes has been observed [31]. The authors speculated that PCV13, as the vaccine with the highest valence (6 serotypes added), has less potential for replacement than PCV7, and the results may depend on the degree of invasiveness and the prevalence of non-vaccine serotypes in the carriers.

Consistent with other studies [19,32], after switching to PCV13, we have observed no increase in the number of cases caused by non-PCV13 serotypes (Fig. 1). Serotypes 8 and 22 F were the most frequent among non-PCV13 serotypes. Serotype 8 showed a significant decreasing trend from one study period to the next, while serotype 22F showed an increase, although not significant. The significant increase in the proportion of the population covered by PPSV23 in addition to other changes such as modification of antibiotic policy or the natural evolution of pneumococcal populations may have conditioned these results. Non-vaccine serotypes represented 29.5% of the total and 23.6% in the period 2011–2014. They were also correlated with a slightly higher than average mortality rate and a complication rate similar to that of the most prevalent serotypes.

Nevertheless, our results only evaluate the early impact, so a longer-term study is needed to rule out serotype replacement. Monitoring of the evolution of serotype replacement will be necessary in the coming years, when greater vaccine coverage in children can be expected. Since 2015 vaccine coverage among infants/young children in our region has rapid increased all the way up to 95% in 2017. [33]

Our study has some limitations. We only included cases of BPP that had been hospitalized at both Respiratory Services, hence incidence rates could not be calculated. Since PCV13 is not offered for free under the Spanish public health system, we only have an estimate of the data regarding vaccine coverage in the pediatric population [7], with data based on reports from the vaccine manufacturer, which do not specify whether the patient completed the vaccination schedule, a major shortcoming. Further, our findings reflect only the early impact of PCV13, but longer term follow-up is required to assess the full impact of this vaccine.

The study was conducted in a limited geographical area, and hence, it is not necessarily representative of the situation across Spain. It is, however, worth noting that this was a prospective study and based on a cohort from two hospitals covering a large population, with detailed microbiological information and clinical follow-up for all patients admitted for BPP.

Our findings show that 14 years after the introduction of childhood PCV immunization in our country, mainly with private fund-

ing and with an estimated uptake of around 61% [7], 70.5% of BPP in adults is still being caused by PCV13 serotypes. This indicates that among adults diagnosed with BPP, a significant proportion continues to be due to vaccine serotypes, and suggests that herd protection from "current infant vaccination" levels is not enough to protect adults. Nevertheless, our results only evaluate the early indirect effects of PCV13 introduction among children on BPP among adults. Future long-term studies will help determine if greater declines in vaccine-type BPP will be seen with higher infant vaccine coverage levels, and also the presence and magnitude of serotype replacement.

Author contributions

PPE, AU, LAR, RZ, and JMQ contributed to the study design. PPE, AU AA, LS, APM, RA, and AB conducted the study and contributed to the interpretation of data and PPE, JMQ, AB, RZ to data analysis. PPE, AU, RZ, LAR, and JMQ prepared the manuscript and critically revised the content. All authors approved the version submitted for publication.

Declaration of Competing Interest

Dr. Pedro P. España and Dr. Ane Uranga report a grant to their Institution by Pfizer SLU, Madrid, Spain for another investigation. The sponsor had no role in this study. The remaining authors have no conflicts of interest to declare.

Acknowledgements

We would like to thank the translation and editorial assistance provided by Ideas Need Communicating Language Services, through the translation and edition service of the Basque Foundation for Health Innovation and Research (BIOEF).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2019.05.052.

References

[1] Welte T, Torres A, Nathwani D. Clinical and economic burden of community-acquired pneumonia among adults in Europe. Thorax 2012;67(1):71–9. Jan.
[2] Said MA, Johnson HL, Nonyane BA, Deloria-Knoll M, O'Brien KL; AGEDD Adult Pneumococcal Burden Study Team, Andreo F, Beovic B, Blanco S, Boersma WG, Boulware DR, Butler JC, Carratalà J, Chang FY, Charles PG, Diaz AA, Domínguez J, Ehara N, Endeman H, Falcó V, Falguera M, Fukushima K, Garcia-Vidal C, Genne D, Guchev IA, Gutierrez F, Hernes SS, Hoepelman AI, Hohenthal U, Johansson N, Kolek V, Kozlov RS, Lauderdale TL, Marekvić I, Masiá M, Matta MA, Miró Ò, Murdoch DR, Nuermberger E, Paolini R, Perelló R, Snijders D, Plečko V, Sordé R, Strålin K, van der Eerden MM, Vila-Corcoles A, Watt JP . Estimating the burden of pneumococcal pneumonia among adults: a systematic review and meta-analysis of diagnostic techniques. PLoS One. 2013;8(4):e60273.
[3] Chalmers JD, Campling J, Dicker A, Woodhead M, Madhava H. A systematic review of the burden of vaccine preventable pneumococcal disease in UK adults. BMC Pulm Med 2016;16(1):77. May 11.
[4] Capelastegui A, Zalacain R, Bilbao A, Egurrola M, Iturriaga LA, Quintana JM, Gomez A, Esteban C, España PP. Pneumococcal pneumonia: differences according to blood culture results. BMC Pulm Med 2014;14(1). https://doi.org/10.1186/1471-2466-14-128.
[5] Kalin M, Ortqvist A, Almela M, Kalin M, Ortqvist A, Almela M, et al. Prospective study of prognostic factors in community-acquired bacteremic pneumococcal disease in 5 countries. J Infect Dis 2000;182:840–7.
[6] Kim JO, Romero-Steiner S, Sørensen UB, Blom J, Carvalho M, Barnard S, et al. Relationship between cell surface carbohydrates and intrastrain variation on opsonophagocytosis of Streptococcus pneumoniae. Infect Immun 1999;67(5):2327–33. May.
[7] Gervaix A, Ansaldi F, Brito-Avô A, Azzari C, Knuf M, Martinón-Torres F, Tuerlinckx D, Htar MT, Syrogiannopoulos GA. Pneumococcal vaccination in Europe: schedule adherence. Clin Ther 2014;36(5). 802e1 12 May.
[8] Chalmers JD, Campling J, Dicker A, et al. A systematic review of the burden of vaccine preventable pneumococcal disease in UK adults. BMC Pulm Med 2016;16:77.
[9] Centers for Disease Control and Prevention (CDC). Licensure of 13-valent pneumococcal conjugate vaccine for adults aged 50 years and older. MMWR Morb Mortal Wkly Rep. 2012 Jun 1;61(21):394-5.
[10] Grau I, Ardanuy C, Calatayud L, Schulze MH, Liñares J, Pallares R. Smoking and alcohol abuse are the most preventable risk factors for invasive pneumonia and other pneumococcal infections. Int J Infect Dis 2014;25:59–64. Aug.
[11] Menéndez R, Torres A, Aspa J, Capelastegui A, Prat C, Rodríguez de Castro F. Sociedad española de neumología y cirugía torácica. community acquired pneumonia. new guidelines of the spanish society of chest diseases and thoracic surgery (SEPAR)]. Arch Bronconeumol 2010;46(10):543–58. Oct.
[12] Fine MJ, Auble TE, Yealy DM, Hanusa BH, Weissfeld LA, Singer DE, et al. A prediction rule to identify low-risk patients with community-acquired pneumonia. N Engl J Med 1997;336:243–50.
[13] Marimon JM, Ercibengoa M, Santacatterina E, et al. Single-step multiplex PCR assay for determining 92 pneumococcal serotypes. J Clin Microbiol 2016;54:2197–200.
[14] Weinberger DM, Harboe ZB, Sanders EA, Ndiritu M, Klugman KP, Rückinger S, et al. Association of risk with death due to pneumococcal pneumonia: a meta-analysis. Clin Infect Dis 2010;51:692–9.
[15] Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, Reingold A, Thomas A, Schaffner W, Craig AS, Smith PJ, Beall BW, Whitney CG, Moore MR. Active bacterial core surveillance/emerging infections program network. sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. J Infect Dis 2010;201(1):32–41. Jan 1.
[16] Waight PA, Andrews NJ, Ladhani SN, et al. Effect of the 13-valent pneumococcal conjugate vaccine on invasive pneumococcal disease in England and Wales 4 years after its introduction: an observational cohort study. Lancet Infect Dis 2015;15:535–43.
[17] Moore MR, Link-Gelles R, Schaffner W, et al. Effect of use of 13-valent pneumococcal conjugate vaccine in children on invasive pneumococcal disease in children and adults in the USA: analysis of multisite, population-based surveillance. Lancet Infect Dis 2015;15:301–9.
[18] Galanis I, Lindstrand A, Darenberg J, Browall S, Nannapaneni P, Sjöström K, et al. Effects of PCV7 and PCV13 on invasive pneumococcal disease and carriage in Stockholm Sweden. Eur Respir J 2016;47(4):1208–18. Apr.
[19] Azzari C, Cortimiglia M, Nieddu F, Moriondo M, Indolfi G, Mattei R, et al. Pneumococcal serotype distribution in adults with invasive disease and in carrier children in Italy: should we expect herd protection of adults through infants' vaccination? Hum Vaccin Immunother 2016;12(2):344–50.
[20] Aliberti S, Mantero M, Mirsaeidi M, Blasi F. The role of vaccination in preventing pneumococcal disease in adults. Clin Microbiol Infect 2014;20 (Suppl 5):52–8. May.
[21] Torres A, Rodríguez-Créixems M, Grau I, Molinos L, Llinares P, De la Cruz JL, Rajas O, Alfageme I, Salavert M, Fenoll A, Liñares J, Cifuentes I. Underlying clinical conditions and invasive pneumococcal disease (IPD) in adults in Spain (ODIN study, 2010–2012). Abstract 5051. European Respiratory Society (ERS) Annual Congress 2013, Barcelona, Spain, 7–11 September 2013, 2013.
[22] Ardanuy C, Marimón JM, Calatayud L, et al. Epidemiology of invasive pneumococcal disease in older people in Spain (2007–2009): Implications for future vaccination strategies. PLoS One 2012;7.
[23] Torres A, Blasi F, Dartois N, et al. Which individuals are at increased risk of pneumococcal disease and why? Impact of COPD, asthma, smoking, diabetes, and/or chronic heart disease on community-acquired pneumonia and invasive pneumococcal disease. Thorax 2015;70:984–9.
[24] van Hoek AJ, Andrews N, Waight PA, Stowe J, Gates P, George R, Miller E. The effect of underlying clinical conditions on the risk of developing invasive pneumococcal disease in England. J Infect 2012;65(1):17–24. Jul.
[25] González-Romo F, Picazo JJ, García Rojas A, Labrador Horrillo M, Barrios V, Magro MC, Gil Gregorio P, de la Cámara R, Rodríguez A, Barberán J, Botía Martínez F, Linares Rufo M, Jimeno Sanz I, Portolés JM, Sanz Herrero F, Espinosa Arranz J, García-Sánchez V, Galindo Izquierdo M, Mascarós E. Consensus document on pneumococcal vaccination in adults at risk by age and underlying clinical conditions. 2017 Update. Rev Esp Quimioter 2017;30 (2):142–68. Apr.
[26] Burgos J, Falcó V, Borrego A, Sordé R, Larrosa MN, Martinez X, Planes AM, Sánchez A, Palomar M, Rello J, Pahissa A. Impact of the emergence of non-vaccine pneumococcal serotypes on the clinical presentation and outcome of adults with invasive pneumococcal pneumonia. Clin Microbiol Infect 2013 (4):19385–91. Apr.
[27] Bewick T, Sheppard C, Greenwood S, Slack M, Trotter C, George R, et al. Serotypes associated with the development of pneumococcal para-pneumonic effusion in adults. Eur Respir J 2013 Sep;42(3):733–41.
[28] Moreira M, Castro O, Palmieri M, Efklidou S, Castagna S, Hoet B. A reflection on invasive pneumococcal disease and pneumococcal conjugate vaccination coverage in children in Southern Europe (2009–2016). Hum Vaccin Immunother 2017;13(6):1–12. Jun 3.
[29] Bonten MJ, Huijts SM, Bolkenbaas M, Webber C, Patterson S, Gault S, et al. Polysaccharide conjugate vaccine against pneumococcal pneumonia in adults. N Engl J Med 2015;372(12):1114–25. Mar 19.
[30] Webber C, Patton M, Patterson S, Schmoele-Thoma B, Huijts S, Bontene M. For the CAPiTA study group. exploratory efficacy endpoints in the community acquired penumonia immunization trial in adults (CAPITA). Vaccine 2017;35:1266–72.

[31] Guevara M, Ezpeleta C, Gil-Setas A, Torroba L, Beristain X, Aguinaga A, García-Irure JJ, Navascués A, García-Cenoz M, Castilla J. Working Group for surveillance of the pneumococcal disease in navarre. reduced incidence of invasive pneumococcal disease after introduction of the 13-valent conjugate vaccine in Navarre, Spain, 2001-2013. Vaccine 2014;32(22):2553–62. May 7.
[32] Pletz MW, Ewig S, Rohde G, Schuette H, Rupp J, Welte T, et al. CAPNETZ Study Group. impact of pneumococcal vaccination in children on serotype distribution in adult community-acquired pneumonia using the serotype-specific multiplex urinary antigen detection assay. Vaccine 2016;34(20):2342–8. Apr 29.
[33] Descriptive epidemiology of invasive pneumococcal disease in Bizkaia 2017, published in 2018. <http://www.osakidetza.euskadi.eus/r85-pkvigi05/es/contenidos/informacion/memorias_epidemiologica/es_def/index.shtml>.