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ORIGINAL ARTICLE

Invasive pneumococcal disease in children under 60 months before and after availability of 13-valent conjugate vaccine☆

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KEYWORDS
Pneumococcal conjugate vaccine;
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Abstract
Background: Invasive pneumococcal disease (IPD) is the most important bacterial infection in young children, and the introduction of pneumococcal conjugate vaccines has changed its presentation. This study compared the incidence, characteristics and serotype distribution of IPD before and after the introduction of the pneumococcal conjugate vaccine (PCV13).
Methods: Prospective enrolment of patients with IPD aged less than 60 months and admitted to either of 2 tertiary care hospitals between January 2007 and December 2009 (pre-PCV13 period) and January 2012 and June-2016 (PCV13 period).

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Results: We identified 493 cases, 319 in the pre-PCV13 period and 174 in the PCV13 period. The incidence of IPD decreased from 89.7 to 34.4 casos per 100 000 habitantes (−62%; *P* < .001). This decrease was observed in all forms of disease except necrotising pneumonia (increase from 0.8 to 3.7 casos/100 000 population). There was a significant reduction in all serotypes included in the PCV13 and not included in the PCV7. We did not find significant differences in length of stay, mortality or the frequency of sequelae between both periods, but in the PCV13 period, the length of stay in the paediatric intensive care unit and the duration of mechanical ventilation were longer (*P* = .00). The incidence of serotype 3 decreased from 10.4 to 6.9 casos per 100 000 population, although it was the serotype involved most frequently in patients with severe disease.
Conclusions: After the introduction of the PCV13, there has been a significant decrease in IPD cases. Serotype 3 continues to be an important cause of severe IPD.

PALABRAS CLAVE
Vacuna antineumocócica conjugada VNC;
Enfermedad neumocócica invasiva;
Streptococcus pneumoniae

Enfermedad neumocócica invasiva en niños menores de 60 meses, antes y después de la introducción de la vacuna conjugada 13-valente

Resumen
Introducción: La enfermedad neumocócica invasiva (ENI) es la infección bacteriana más relevante en niños pequeños y la introducción de las vacunas antineumocócicas conjugadas (VNC) ha cambiado su presentación clínica. En este estudio se analizaron los cambios en la incidencia, características clínicas y distribución de serotipos en los casos de ENI antes y después de la disponibilidad de la VNC13.
Métodos: Se incluyeron prospectivamente pacientes con ENI menores de 60 meses ingresados en dos hospitales pediátricos terciarios desde enero de 2007 a diciembre de 2009 (periodo pre-VNC13) y de enero de 2012 a junio de 2016 (período VNC13).
Resultados: Se identificaron 493 casos, 319 en el período pre-VNC13 y 174 en el período VNC13. La incidencia de ENI disminuyó de 89,7 a 34,4 casos por 100 000 habitantes (−62%, *p* < 0,001). Esta disminución de la incidencia se dio por igual en todas las presentaciones clínicas de la enfermedad excepto en la neumonía necrotizante (aumentó de 0,8 a 3,7 casos x 100 000 habitantes). Todos los serotipos incluidos en la VNC13 pero no incluidos en la VNC7 disminuyeron significativamente. No se encontraron diferencias significativas en la estancia hospitalaria, muerte y/o secuelas entre ambos períodos, aunque durante el período VNC13, los pacientes requirieron más días en la unidad de cuidados intensivos pediátricos y de ventilación mecánica (*p* = 0,00). La incidencia del serotipo 3 disminuyó de 10,4 a 6,9 casos x 100 000 habitantes, aunque fue el serotipo más frecuente en los pacientes con un cuadro clínico grave.
Conclusiones: luego de la introducción de la VNC13 se ha producido una disminución significativa de los casos de ENI. El serotipo 3 sigue siendo una causa importante de casos graves de enfermedad neumocócica invasiva.

Introduction

Streptococcus pneumoniae is a gram-positive bacterium with more than 95 identified capsular serotypes[1]. Infection with this pathogen is a major cause of morbidity and mortality in both adults and children worldwide, with a wide clinical spectrum that ranges from asymptomatic colonization to mucosal disease, and invasive infection (involving previously sterile sites)[2,3]. Before the introduction of the pneumococcal conjugate vaccine, *S. pneumoniae* caused between 8% and 12% of all deaths in children under 5 years, amounting to an estimated 1 million deaths per year worldwide. In 2008, the mortality was estimated at 500 000 deaths per year worldwide[4–6].

Invasive pneumococcal disease (IPD) is most prevalent in the very young and the elderly, especially in children under 5 years. The most frequent presentations are pneumonia, meningitis and bacteraemia. The introduction of pneumococcal conjugate vaccines has had a significant impact on IPD in terms of disease incidence and the serotype distribution. After the introduction of the heptavalent pneumococcal conjugate vaccine (PCV7) in the United States, there was a dramatic decline in the incidence of IPD. However, in our region there was a significant increase in the incidence of IPD

caused by non-PCV7 serotypes, a slight reduction in the rate of IPD caused by PCV7 serotypes and emergence of previously stable virulent clones of non-PCV7 serotypes[2,7–9]. With the aim of improving vaccine coverage against non-PCV7 serotypes, the 13-valent pneumococcal conjugate vaccine (PCV13) was approved in 2010 for active immunization of children aged 6 weeks to 5 years. Although the Advisory Committee on Vaccines of the Asociación Española de Pediatría (Spanish Association of Pediatrics, AEP) has recommended routine administration of conjugate pneumococcal vaccines (PCV7 in the 2003–2010 period and PCV13 once it had been authorised/introduced), at the time of this writing these vaccines were not funded by the public health system of Catalonia except for children with selected risk factors, and therefore their administration depended on the judgment of the paediatrician and the willingness of families. The pneumococcal vaccine coverage in Catalonia in children under 60 months has been estimated at 50% in the pre-PCV13 period and 63% in the PCV13 period, based on previous studies conducted by our group[9–11].

The aim of the present study was to compare the incidence and describe epidemiological variables, the clinical presentation, current trends and serotype distribution for IPD among children before and after the introduction of the PCV13 in Catalonia, Spain, a region where this vaccine was not included in the routine vaccination schedule.

Methods

Sample and definitions

We conducted a prospective study in patients with IPD aged less than 60 months and admitted to either of 2 tertiary care hospitals in Barcelona (Catalonia, Spain) over 6 years. The study period was divided into a pre-PCV13 period (January 2007 to December 2009) and a PCV13 period (January 2012 to June 2016). The participating hospitals were the Hospital Sant Joan de Déu and the Hospital Vall d'Hebron, 2 referral children's hospitals corresponding to more than 30% of the total paediatric admissions in Catalonia, a region with a population of 7 500 000 inhabitants of who more than 400 000 are children aged less than 5 years[12]. After the introduction of the PCV13 in 2010, the vaccine coverage in children under 60 months in Catalonia was estimated at 55% in the 2012–2013 period and 78% in 2015[12,13].

We defined case of IPD as the presence of clinical manifestations of infection with detection of *S. pneumoniae* in any normally sterile body fluid[2,10,12]. We defined microbiological detection as isolation of a *S. pneumoniae* strain in culture and/or detection by real-time polymerase chain reaction (RT-PCR) of *ply* and/or *lyta* genes and an additional capsular gene of *S. pneumoniae*.

We obtained the vaccination status from the personal vaccination card or primary care records of the patients. The PCV13-PCV7 vaccination schedule in Catalonia follows a 3 + 1 scheme, with administration of 3 doses in the first 6 months of life (at ages 2, 4 and 6 months) followed by a booster dose at age 12–15 months. We defined PCV vaccine failure as recommended by the Council for the International Organizations of Medical Sciences and the World Health Organization (WHO) Working Group as illness in a correctly vaccinated individual[13–15].

Confidentiality and ethical considerations

The study did not involve performance of diagnostic tests or collection of samples in any participant besides those required by routine care. The protocol adhered to the principles of the Declaration of Helsinki and current legislation on international human rights, biomedicine and the protection of personal data and was approved by the Clinical Research Ethics Committee of the Fundació Sant Joan de Déu. We obtained signed informed consent from the parents or legal guardians of all participants (cases and controls). All data were kept confidential and data collection from records was anonymised.

Data collection

For each patient, we collected general and clinical data at hospital admission, discharge and 6 months post discharge. The epidemiological variables included age, sex, *S. pneumoniae* vaccination status (when documented), comorbidities (with categorization into 2 risk groups based on the criteria of the American Academy of Pediatrics[13–15]), attendance to childcare centre, antibiotic treatment and/or respiratory infection before the diagnosis of IPD, history of breastfeeding, clinical presentation of IPD and past medical history.

The clinical variables included the clinical presentation, length of stay, intensive care unit (ICU) admission, complications, antibiotherapy at admission and discharge, death and presence of sequelae at 180 days post discharge. We also collected microbiological data on the identified *S. pneumoniae* serotypes and antimicrobial susceptibility to penicillin/cefotaxime.

Microbiological testing

All pneumococcal isolates were identified by the same microbiological methods throughout the study period, including the optochin susceptibility test and an antigen test that targets the capsular polysaccharide (Slidex pneumo-kit, bioMérieux, Marcy-l'Étoile, France). Detection of *S. pneumoniae* DNA was performed by RT-PCR assays with previously described methods, including amplification of the pneumolysin gene (*ply*) or the autolysin gene (*lyta*)[16,17]. Serotyping of the strains isolated by culture was performed with the Quellung reaction at the Centro Nacional de Microbiología (Majadahonda, Spain). In cases diagnosed based solely on the RT-PCR test, previously validated methods were applied for detection of pneumococcal serotypes[18,19]. In the pre-PCV13 period, the PCR protocol included detection of DNA in the conserved *wzg* capsular gene and other genes of *S. pneumoniae* selected to distinguish 24 serotypes (1, 3, 4, 5, 6A/C, 6B/D, 7F/A, 8, 9V/A/N/L, 14, 15B/C, 18C/B, 19A, 19F/B/C, 23A and 23F). In the PCV13 period, the protocol had been improved and allowed identification of 40 serotypes in samples with a high bacterial DNA load, defined by a RT-PCR cycle threshold of 30 or less (1, 2, 3, 4, 5, 6A/6B, 6C, 7C/(7B/40), 7F/7A, 9N/9L, 9V/9A,

10A, 10F/(10C/33C), 11A/11D, 12F/(12A/44/46), 13, 16F, 17F, 18/(18A/18B/18C/18F), 19A, 19F, 20(20A/20B), 21, 22F/22A), 23A, 23B, 24/(24A/24B/24F), 31, 34, 35A/(35C/42), 35B, 35F/47F, 38/25F, 39)[18,20]. We defined susceptibility to penicillin and third generation cephalosporins applying the 2015 meningeal breakpoints of the Clinical Laboratory Standards Institute (CLSI) using American Type Culture Collection 49619 (serotype 19F) as the control. We defined resistance to penicillin as a minimum inhibitory concentration (MIC) of 0.12 μg/mL or greater and resistance to cefotaxime as a MIC of 2 μg/mL or greater[21].

Statistical analysis

We summarised categorical variables as absolute frequencies and percentages and continuous variables as mean ± standard deviation (SD). To compare categorical variables between groups, we used the Pearson chi square or Fisher exact test as applicable, and we used the non-parametric Mann–Whitney *U* test to assess differences in continuous variables. We defined statistical significance as a *p*-value of 0.05 or less. The incidence of IPD, defined as the number of cases per 100 000 inhabitants, was calculated using the annual estimates in the paediatric population obtained from the Department of Statistics of Catalonia and the proportion of the total hospitalizations in children under 5 years managed by the 2 participating hospitals[22]. The statistical analysis was performed with the software packages SPSS version 18.0 (IBM Corp) and OpenEpi 3.0[23].

Results

During the study period, 493 cases of IPD were detected, 319 in the pre-PCV13 period and 174 in the PCV13 period. A serotype had been documented in 395 of the total cases (245 in the pre-PCV13 period and 150 in the PCV13 period). Table 1 presents the analysis of the baseline characteristics. We found differences in terms of breastfeeding, attendance to childcare centre or school, previous history of respiratory infection, risk factors for pneumococcal disease, microbiological diagnosis and the sites from which positive samples were obtained.

Vaccination status

In the first period, 131 cases (44.7%) occurred in patients correctly vaccinated with PCV7 for their age and 3 of the cases were caused by PCV7 serotypes (vaccine failure rate, 2.3%). In the second period, 49 cases (32%) occurred in patients correctly vaccinated with PCV13 for their age, 12 of the cases were caused by PCV13 serotypes (vaccine failure rate, 24%, with 10 cases [83.3%] caused by serotype 3). The supplemental table summarises the characteristics of fully vaccinated children with IPD.

Incidence

Table 2 shows the incidence of IPD overall and by clinical presentation. In the pre-PCV13 period, the incidence of IPD increased between 2007 and 2009: 76.2 cases per 100 000 inhabitants in 2007, 82.1 cases per 100 000 inhabitants in 2008 and 109.8 cases per 100 000 inhabitants in 2009. In the PCV13 period, the incidence decreased gradually from 39.6 cases per 100 000 inhabitants in 2012 to 29.7, 32.5, 36.2 and 33 cases per 100 000 inhabitants in 2013, 2014, 2015 and 2016, respectively.

The incidence of IPD after the introduction of the PCV13 vaccine decreased from 89.7 cases per 100 000 inhabitants to 34.4 cases per 100 000 inhabitants, corresponding to a −62% reduction (95% confidence interval [CI], −69% to −54%; $P < .001$).

Table 3 summarises the changes in the frequency of PCV13 serotypes. The frequency of serotypes 3, 1, 19A, 7FA, 5, 6A/C and 19F decreased significantly while changes in the remaining PCV13 serotypes were not significant.

Cases of IPD in the pre-PCV13 period were mainly caused by PCV13 serotypes. After the introduction of the PCV13, we observed a significant reduction in IPD caused by PCV13 serotypes overall, with no evidence of serotype replacement.

Clinical presentation and outcomes

The clinical presentation was different in the 2 periods, with a decrease in the incidence of pneumonia (from 71.3–23.7 cases/100 000 inhabitants; $P < .001$), meningitis (from 8.1–3.5 cases/100 000 inhabitants; $P = .002$) and occult bacteraemia (from 7.0–3.5 cases/100 000 inhabitants; $P = .012$). The incidence of pneumonia with empyema decreased from 47.7–11.4 cases per 100 000 inhabitants ($P < .001$) of the total cases of pneumonia. There was an important increase in the incidence of necrotizing pneumonia during the study period (from 0.8 to 3.7 cases/100 000 inhabitants; $P = .004$), which corresponded to a 340% increase (95% CI, 30%–1400%) between the 2 periods. There were no significant changes in the incidence of septic shock and osteoarticular infection during the study period (Table 2).

We did not find statistically significant differences in the mean length of stay, mortality and frequency of sequelae between both periods, although in the PCV13 period patients had longer stays in the paediatric intensive care unit and required more days of mechanical ventilation ($P = .00$) (Table 1).

Serotypes, molecular testing and antibiotic susceptibility

Serotyping was performed in 466 (94.5%) of the total cases of IPD, 297 (94%) in the first period and 169 (97.1%) in the second period. The most frequently identified serotypes were 1 (62; 20.9%), 19A (47; 15.8%) and 3 (37; 12.5%) in the pre-PCV13 period and 3 (35; 20.7%), 1 (19; 11.2%) and 19A (16; 9.5%) in the PCV13 period. The PCV13 serotypes caused 209 (70.3%) of the 297 cases in the pre-PCV13 period, while in the PCV13 period these serotypes caused 98 (57.9%) of the 169 IPD cases (Table 3).

There was a reduction in the incidence of IPD caused by PCV13 serotypes. The largest decreases corresponded to serotypes 1, 19A, 7F/A and 5. The proportion of cases

Table 1 Baseline characteristics, microbiological diagnosis and outcomes.

	Pre-PCV13 period *n* = 319	PCV13 period *n* = 174	*P*
Baseline characteristics			
Age, mean ± SD (months)	29.6 ± 15.7	27.5 ± 16.2	.16
≤24 months, *n* (%)	129 (40.5%)	82 (47.1%)	.31
25–59 months, *n* (%)	190 (59.5%)	92 (52.8%)	
Sex			.06
Male, *n* (%)	170 (53.3%)	108 (62.1%)	
Female, *n* (%)	149 (46.7%)	66 (37.9%)	
Place of birth, *n* (%)			.37
Spain	298 (93.4%)	164 (94.3%)	
Outside Spain	21 (6.6%)	10 (5.7%)	
Time of hospitalization, *n* (%)			
January–March	106 (33.2%)	65 (38%)	.35
April–June	63 (19.7%)	46 (26.4%)	.08
July–September	24 (7.5%)	9 (5.2%)	.18
October–December	126 (39.5%)	54 (31%)	.06
Underlying disease, *n* (%)	18 (6.1%)	8 (5.3%)	.71
Risk Group 2*	4 (1.3%)	10 (5.7%)	.00
Risk Group 1	0	0	–
Breastfeeding, *n* (%)	224 (73.4%)	142 (81.6%)	.01
Attendance to childcare or school, *n* (%)	244 (79%)	111 (64.5%)	.00
Respiratory infection in previous month, *n* (%)	142 (45.8%)	98 (56.3%)	.01
Antibiotic treatment in previous month, *n* (%)	49 (15.8%)	25 (14.4%)	.15
Microbiological diagnosis			
Identification of *S. pneumoniae*, *n* (%)			
Only through bacterial culture	54 (16.9%)	46 (26.4%)	.01
Only through PCR	206 (64.6%)	84 (48.3%)	.00
Through both culture + PCR	59 (18.5%)	44 (25.3%)	.07
Site of positive samples, *n* (%)			
Blood	103 (32.3%)	86 (49.4%)	.00
Pleural fluid	181 (56.7%)	66 (37.9%)	.00
Cerebrospinal fluid	29 (9.1%)	15 (8.6%)	.46
Joint fluid	5 (1.6%)	4 (2.3%)	.33
Mastoid	0 (0%)	2 (1.1%)	.04
Other	1 (0.2%)	1 (0.7%)	.26
Clinical outcomes			
Length of stay, mean ± SD (days)	10.8 ± 7.5	12.2 ± 9.6	.07
ICU admission, *n* (%)	43 (13.5%)	41 (23.7%)	.00
ICU length of stay, mean ± SD (days)	5.5 ± 6	6.9 ± 9	.40
Mechanical ventilation, *n* (%)	4 (1.3%)	10 (5.7%)	.00
Death, *n* (%)	4 (1.3%)	2 (1.2%)	.93
Sequelae post discharge, *n* (%)	33 (10.3%)	17 (9.9%)	.39

ICU, intensive care unit; PCR, polymerase chain reaction; PCV13, 13-valent pneumococcal conjugate vaccine; SD, standard deviation.
* Risk groups defined based on the criteria of the American Academy of Pediatrics[15].

caused by the main pathogenic serotypes also changed significantly. The proportion of cases of IPD caused by serotype 1 decreased from 20.9%–11.2% in the PCV13 period ($P < .001$), while cases caused by serotype 3 increased from 11.5%–20.7% ($P < .01$).

When it came to severe IPD, serotype 3 was the most frequent serotype involved patients that required ICU admission (15.2%), required mechanical ventilation (33%) with prolonged lengths of stay (27.4%), that developed complications during the stay (20.7%) and with sequelae at discharge (20.8%) (Table 4).

We did not find statistically significant differences in the frequency of strains that were not susceptible to penicillin or cefotaxime (Table 5).

Discussion

Although the vaccine coverage in our region is not high, the incidence of invasive pneumococcal disease in children under 60 months has decreased significantly after the introduction of PCV13. The general characteristics of the population in both periods were similar.

Table 2 Incidence by clinical presentation.

	Pre-PCV13 period		PCV13 period			
	Cases, *n* (%)	Incidence*	Cases, *n* (%)	Incidence*	Change, % (95% CI)	*P*
Incidence of IPD*	319 (64.7%)	89.7	174 (35.3%)	34.4	−62% (−69% to −54%)	<.001
Pneumonia	254 (79.6%)	71.3	120 (69%)	23.7	−67% (−74% to −59%)	<.001
Pneumonia with empyema	170 (53.3%)	47.7	58 (33.3%)	11.4	−76% (−83% to −68%)	<.001
Necrotising pneumonia	3 (0.9%)	0.8	19 (10.9%)	3.7	340% (30% to 1400%)	.004
Meningitis	29 (9.1%)	8.1	18 (10.3%)	3.5	−57% (−76% to −22%)	.002
Occult bacteraemia	25 (7.8%)	7.0	18 (10.3%)	3.5	−50% (−73% to −8%)	.012
Septic shock	3 (0.9%)	0.8	6 (3.4%)	0.1	−40% (−65% to 3058%)	.313
Osteoarticular infection	6 (1.9%)	1.6	7 (4%)	1.3	−18% (−73% to 140%)	.361
Mastoiditis	0 (0%)	0	5 (2.9%)	0.9	–	.030
Cellulitis	2 (0.7%)	0.56	0 (0%)	0	–	.004

CI, confidence interval; IPD, invasive pneumococcal disease; PCV13, 13-valent pneumococcal conjugate vaccine.
* Cases per 100 000 inhabitants.

Pneumonia, meningitis and occult bacteraemia accounted for more than 90% of cases of IPD in both groups. We observed a significant decrease in the cases of pneumonia complicated with empyema associated with a shift in the distribution of serotypes that caused IPD in the PCV13 period (decline of serotype 1), which was consistent with the literature[24–26], while cases of necrotising pneumonia increased substantially from 0.84 to 3.3 cases per 100 000 inhabitants. Although length of stay and mortality did not change with the introduction of the PCV13, we found a relative increase in severity reflected in an increase in the proportion of patients requiring admission to the ICU, requiring ventilation or presenting with septic shock. It is worth noting that there was also a significant increase in the proportion of children with risk factors. We assume that the changes in clinical presentation were due to the impact of vaccination with the PCV13, which was associated with an evident shift in the serotypes causing IPD. In the PCV13 period, opportunistic serotypes, or serotypes with low invasive disease potential, have caused disease with a significantly greater frequency in patients with a higher rate of comorbidities, which may in turn be associated with an increased risk of complications and mortality[27,28]. We believe that the increase in the proportion of cases caused by serotype 3 could have an important impact on the incidence of necrotising pneumonia, we observed an increase in both the absolute and relative frequency of this form of disease between the 2 periods (from 3 [0.8%] to 19 [10.9%]) despite a decrease in overall incidence, which suggests that there are other factors at play in the increase in necrotising pneumonia, such as an increased proportion of patients with comorbidities in the PCV13 period.

Overall, the use of pneumococcal DNA detection by PCR increased the frequency of cases of IPD with microbiological confirmation, as previously reported[29]. However, in the PCV13 period there was a lesser increase of cases with detection by PCR only. This was probably due to the decline in cases manifesting with empyema, as pneumococcal PCR is significantly more sensitive in samples of pleural fluid (where a high bacterial load is expected) compared to blood, where the bacterial load is lower.

There was an overall decrease in the incidence of IPD caused by PCV13 serotypes, with a decline in serotype 1 from being the most frequent (20.9%) in the pre-PCV13 period to accounting for only 11.2% of IPD cases in the PCV13 period. Although we observed a significant decrease in the incidence of IPD caused by serotype 3, this serotype became the most prevalent in the PCV13 period, causing 20.9% of IPD cases. This proportional increase can be explained by the failure rate of the PCV13 vaccine for serotype 3 in our study, a phenomenon previously reported by our group and other authors[12,30,31]. The causes of serotype 3 vaccine failure are not well understood, but are probably related to the low immunogenicity of the vaccine for this particular serotype[12].

Serotype 3 is the most frequent serotype, especially in the most severe cases, such as those requiring ICU admission, mechanical ventilation or prolonged hospitalization and those with complications during the hospital stay or sequelae after discharge. This combination of high incidence and high proportion of severe cases was among the salient results of this study.

One of the limitations of the study was that the molecular techniques used to detect pneumococcal DNA and in serotyping changed during the study. In the first period, we used amplification of the *ply* gene, which has a high sensitivity, and a multiplex PCR assay for detection of 24 serotypes, while in the second period we used amplification of *LytA* gene for pneumococcal detection, which offers a better specificity, and a different molecular assay to detect 40 serotypes. This change in methodology could have played a role in the significant decrease of patients with IPD caused by serotypes not included in the assay.

In conclusion, in the PCV13 period there was a decrease in the incidence of IPD without serotype replacement. We observed changes in epidemiological variables and clinical manifestations, with a proportional increase in cases in children with risk factors and an increase in cases of necrotising pneumonia, with required admission to the ICU and mechanical ventilation in a greater proportion of patients.

We now need to see what happens in upcoming years following the inclusion of the PCV13 in the routine immunisation schedule of Catalonia in July 2016.

Table 3 Distribution of cases of IPD by serotype.

Serotype	Pre-PCV13 period		PCV13 period			
	n (%)	Incidence*	*n* (%)	Incidence*	Change, % (95% CI)	*P*
Serotypes included in the PCV13 vaccine, *n* (%)						
3	37 (12.5%)	10.4	35 (20.7%)	6.9	−34% (−59% to 0%)	.041
1	62 (20.9%)	17.4	19 (11.2%)	3.7	−79% (−88% to −64%)	<.001
19A	47 (15.8%)	13.2	16 (9.5%)	3.1	−77% (−87% to −58%)	<.001
7 FA	21 (7.1%)	5.9	3 (1.8%)	0.5	−90% (−98% to −67%)	<.001
5	9 (3%)	2.5	0 (0%)	0	–	<.001
6A/C	6 (2.0%)	1.6	2 (0.6%)	0.3	−77% (−96% to 10%)	.026
19 F	9 (3%)	2.5	4 (2.4%)	0.7	−69% (−90% to 0%)	.020
14	12 (4.0%)	3.3	14 (8.3%)	2.7	−18% (−63% to 70%)	.307
6B/D	0 (0%)	0	2 (1.2%)	0.3	–	.118
18C/B	1 (0.3%)	0.2	1 (0.6%)	0.1	−30% (−96% to 1000%)	.401
9V/A	2 (0.7%)	0.5	2 (1.2%)	0.3	−30% (−91% to 390%)	.361
23F	3 (1%)	0.8	1 (0.6%)	0.1	−77% (−98% to 120%)	.085
4	0 (0%)	0	1 (0.6%)	0.1	–	.200
Serotypes not included in the PCV13 vaccine, *n* (%)						
10A	2 (0.7%)	0.5	6 (3.6%)	1.1	110% (−58% to 940%)	.17
24F	1 (0.3%)	0.3	4 (2.4%)	0.8	180% (−70% to 2410%)	.17
11A	0 (0%)	0	4 (2.4%)	0.8	–	.04
33F	0 (0%)	0	2 (1.2%)	0.4	–	.11
24A	1 (0.3%)	0.2	2 (1.2%)	0.4	40% −88% to 1452%)	.38
23B	3 (1%)	0.8	3 (1.8%)	0.6	−30% (−86% to 248%)	.33
15A	2 (0.7%)	0.5	2 (1.2%)	0.4	−30% (−91% to 399%)	.36
15C	1 (0.3%)	0.3	1 (0.6%)	0.2	−30% (−96% to 1000%)	.40
22F	1 (0.3%)	0.3	2 (1.2%)	0.4	40% (−88% to 1452%)	.38
38	1 (0.3%)	0.3	2 (1.2%)	0.4	40% (—88% to 1452%)	.38
12F/A/44/46	0 (0%)	0	3 (1.8%)	0.6	–	.07
6C	0 (0%)	0	1 (0.6%)	0.2	–	.20
8	0 (0%)	0	1 (0.6%)	0.2	–	.20
13	0 (0%)	0	1 (0.6%)	0.2	–	.20
15B	0 (0%)	0	1 (0.6%)	0.2	–	.20
16F	0 (0%)	0	1 (0.6%)	0.2	–	.20
31	0 (0%)	0	2 (1.2%)	0.4	–	.11
27	0 (0%)	0	1 (0.6%)	0.2	–	.20
25F	0 (0%)	0	1 (0.6%)	0.2	–	.20
35B	0 (0%)	0	1 (0.6%)	0.2	–	.20
24	0 (0%)	0	2 (1.2%)	0.4	–	.11
9V/A	0 (0%)	0	1 (0.6%)	0.2	–	.20
6A/C	0 (0%)	0	1 (0.6%)	0.2	–	.20
33AF/37	0 (0%)	0	1 (0.6%)	0.2	–	.20
35F	0 (0%)	0	1 (0.6%)	0.2	–	.20
24B	1 (0.3%)	0.3	0 (0%)	0	–	.11
28	1 (0.3%)	0.3	0 (0%)	0	–	.11
Serotypes not included in the assay, *n* (%)						
SNIA	74 (24.9%)	20.7	24 (14.2%)	4.7	−78% (−86% to −64%)	.00

CI, confidence interval; IPD, invasive pneumococcal disease; PCV13, 13-valent pneumococcal conjugate vaccine; SNIA, serotype not included in the serotyping assay.

* Number of cases per 100 000 inhabitants.

Conflicts of interest

Dr de Sevilla MF received grants from the Instituto de Salud Carlos III and fees from Pfizer Inc. while the study was underway. Dr Moraga-Llop has received fees from Pfizer Inc. outside the study period. Dr Campins received fees from Pfizer while the study was underway. Dr Muñoz-Almagro has received grants from Pfizer, bioMérieux, Stat DX and the Instituto de Salud Carlos III and fees from Roche and GSK outside the study period. Dr García-García received grants from Instituto de Salud Carlos III and fees from Pfizer Inc. while the study was underway. The remaining authors had no conflicts of interest to disclose.

Table 4 Distribution of the most prevalent serotypes by clinical outcome.

Outcome	Serotype	Patients, *n* (%)	Outcome	Serotype	Patients, *n* (%)
ICU admission	19A	13 (16.4%)	No ICU admission	1	77 (19.8%)
n = 79	3	12 (15.1%)	*n* = 387	3	60 (15.5%)
	7F	5 (6.3%)		19A	50 (12.9%)
	19F	4 (5.0%)		14	23 (5.9%)
	1	4 (5.0%)		7FA	9(2.3%)
Mechanical	3	4 (33.3%)	No mechanical	1	81 (17.8%)
ventilation	7F	1 (8.3%)	ventilation	3	68 (15%)
n = 12	19A	1 (8.3%)	*n* = 454	19A	62 (13.7%)
	11A	1 (8.3%)		14	25 (5.5%)
	14	1 (8.3%)		19F	12 (2.6%)
Prolonged stay >14	3	26 (27.4%)	Length of stay <14	1	71 (19.1%)
days	19A	16 (16.8%)	days	19A	47 (12.7%)
n = 95	1	10 (10.5%)	*n* = 371	3	46 (12.4%)
	7F	5 (5.3%)		14	21 (5.7%)
	14	5 (5.3%)		19F	10 (2.7%)
Complications	3	66 (20.7%)	Without	19A	20 (14.1%)
n = 324	1	66 (20.7%)	complications	1	14 (9.9%)
	19A	43 (13.3%)	*n* = 142	19F	10 (7.0%)
	14	19 (5.9%)		14	7 (4.9%)
	7FA	10 (3.1%)		10A	6 (4.2%)
Sequelae post	3	10 (20.8%)	Free of sequelae	1	79 (18.8%)
discharge	19A	6 (12.5%)	*n* = 418	3	62 (14.8%)
n = 48	7FA	3 (6.2%)		19A	56 (13.3%)
	14	3 (6.2%)		14	22 (5.2%)
	7F	3 (6.2%)		19F	12 (2.8%)

ICU, intensive care unit.

Table 5 Antimicrobial susceptibility in 208 invasive pneumococcal strains.

	pre-PCV13 period	PCV13 period	*P*
Antibiogram performed, *n* (%)	125 (39.2%)	83 (47.7%)	.08
Antibiotic susceptibility*			
Penicillin MIC, mean ± SD (μg/mL)	0.42 ± 0.79	0.55 ± 1.15	.33
Resistance to penicillin, *n* (%)	41 (34.5%)	41 (47.1%)	.06
Cefotaxime MIC, mean ± SD (μg/mL)	0.26 ± 0.41	0.42 ± 1	.12
Resistance to cefotaxime, *n* (%)	2 (1.7%)	5 (5.7%)	.11

MIC, minimum inhibitory concentration; PCV13, 13-valent pneumococcal conjugate vaccine; SD, standard deviation.
* Susceptibility defined applying CLSI 2015 breakpoints[21].

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fundraising. Juan José (JJ) García-García, PhD: Design and methodology, Expertise, feedback, writing and fundraising.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.anpedi.2021.05.018.

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