Necrotizing pneumonia among italian children in the pneumococcal conjugate vaccine era

NECROTIZING PNEUMONIA AMONG ITALIAN CHILDREN IN THE PNEUMOCOCCAL CONJUGATE VACCINE ERA

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ABSTRACT

Background: Necrotizing pneumonia (NP) is a severe complication of community-acquired pneumonia. The impact of 13-valent pneumococcal conjugate vaccine (PCV13) on the epidemiology of NP in children has not been formally assessed.

Patients and methods: Medical records of children <18 years admitted with NP to two pediatric hospitals in Italy between 2005 through 2019 were retrospectively reviewed. The following 4 periods were defined: 2005-2010 (pre-PCV13), 2011-2013 (early post-PCV13), 2014-2016 (intermediate post-PCV13), and 2017-2019 (late post-PCV13).

Results: Forty-three children (median age, 44 months) were included. Most of them (93%) were previously healthy. No differences in age, sex, season of admission, comorbidity, clinical presentation, or hospital course were identified between pre-PCV13 and post-PCV13 periods. A significant decrease in the rate of NP-associated hospitalizations was found between the early (1.5/1000 admissions/year) and the intermediate (0.35/1000 admissions/year) post-PCV13 period (p=0.001). *Streptococcus pneumoniae* was the most common agent detected in both periods (pre-PCV13: 11/18, 61%; post-PCV13: 13/25, 52%). Serotype 3 was the most common strain in both periods (pre-PCV13: 3/11, 27%; post-PCV13; 4/13, 31%). There were no changes in the bacterial etiology over time, but most patients with *Streptococcus pyogenes* or *Staphylococcus aureus* infection were admitted during the post-PCV13 period.

Conclusions: The hospitalization rate for NP in children decreased a few years after the implementation of PCV13 immunization in Italy. However, an increased trend in admissions was found thereafter. *S. pneumoniae* was the most frequent causal agent in both pre- and post-PCV13 periods. Pneumococcal serotypes were mainly represented by strain 3.

INTRODUCTION

Necrotizing pneumonia (NP) is a severe complication of community-acquired pneumonia.[1] It is characterized by destruction and cavitation of the lung parenchyma ("Emmentaler lung"), and is frequently associated with pleural involvement.[2] Since the first description of NP in childhood,[3] increasing cases have been reported in previously healthy children[4] with special emphasis to clinical,[5] laboratory,[6] pathology,[7] radiology,[8] and therapeutic[2] aspects. *Streptococcus pneumoniae* is the most common agent identified in children with NP. A greater physician's awareness, improved diagnostics, temporal trends or vaccine-induced changes in *S. pneumoniae* serotype prevalence, and the propensity of some strains to cause NP may account for the high incidence of NP over the last twenty years.[9]

The introduction of the 7-valent pneumococcal conjugate vaccine (PCV7, containing serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F) into immunization programs initially led to a declining incidence of pneumonia in childhood,[10,11] but was associated with an increased incidence of empyema,[12,13,14] primarily due to non-vaccine serotypes.[15] Following global replacement of PCV7 with 13-valent PCV (PCV13, containing additional serotypes 1, 3, 5, 6A, 7F, 19A), incidence and hospitalization rates for empyema reduced substantially[16,17,18,19,20] without serotype shift.[21,22,23,24]

The impact of the switch from PCV7 to PCV13 on NP incidence remains unclear. No study has formally evaluated the epidemiology of NP in children before and after PCV13 implementation. Therefore, the aim of our study was to assess the impact of PCV13 on hospitalization rate and etiology of NP in children admitted to two tertiary care pediatric hospitals in Italy over a 15-year period (2005-2019).

PATIENTS AND METHODS

Study design

A retrospective review of medical records of children <18 years hospitalized with NP in two tertiary care pediatric hospitals in Italy (Salesi Children's Hospital, Ancona, and Meyer Children's Hospital, Firenze) between January 2005 through December 2019 was conducted using an electronic database. All the included data were obtained as part of routine clinical activity and were evaluated anonymously. Therefore, a specific approval by the ethical committee was not required.

Data collection and definitions

Clinical information extracted from medical records was registered on a standardized form, which included demographics, time of admission, clinical presentation, comorbidities, pneumococcal immunization status, laboratory results, imaging studies, treatment strategies, hospitalization course and outcome. When the vaccination status was not available in the medical record, the family was contacted by telephone in order to check the child's vaccination card. In Italy, hospital discharges are coded in accordance with the International Classification of Diseases, 9th revision (ICD-9). We included codes for NP (513.0), empyema (510.9) and pleural effusion (511.9). Patients with known risk factors, such as congenital lung abnormalities, cystic fibrosis, primary ciliary dyskinesia, neurological compromise, immunodeficiency, cancer chemotherapy, and those with nosocomial pneumonia or pneumonia due to *Mycobacterium tuberculosis* were excluded.

Diagnosis of NP and parapneumonic effusion/empyema was made as previously described.[21] NP was defined by the combination of clinical symptoms and signs of pneumonia with specific radiological findings (pulmonary consolidation, loss of normal lung architecture, and single or multiple thin-walled parenchymal cavities filled with fluid and/or air) on chest radiograph and/or computed tomography scan. Parapneumonic effusion/empyema was defined as the presence of free pleural fluid on chest radiograph, or loculated pleural fluid on chest ultrasound or computed tomography scan, in association with characteristic biochemical criteria. A patient was considered as having prior antibiotic therapy if he had completed 48 hours of an appropriate antibiotic for the treatment of community-acquired pneumonia. A comorbidity was defined as a chronic medical condition. Annual rates of NP per 1000 admissions were calculated by the number of NP cases per year by the total admissions under

the pediatric internal medicine team (as all patients with possible NP would be initially admitted under this team).

We defined the following 4 periods according to PCV13 implementation: January 2005 to December 2010, pre-PCV13 period, including a transitional period (switch from PCV7 to PCV13 in 2010); January 2011 to December 2013, early post-PCV13 period; January 2014 to December 2016, intermediate post-PCV13 period; and January 2017 to December 2019, late post-PCV13 period.

Vaccine coverage

In Italy, PCV13 was included in a national immunization plan with a 3-dose-schedule (3-5-12 months) in replacement of PCV7 in the last quarter of 2010. No catch-up campaign for children >1 years of age has been carried out since the introduction of PCV13. Mean annual vaccination coverage for PCV13 in Italian target populations in the post-PCV13 era was over 85% in the years 2013-2018; data about national vaccination coverage were not available for the years 2011, 2012 and 2019 (http://www.salute.gov.it/portale/documentazione/p62831.jsp?lingua = italiano&id=20; accessed in June 2020). For our purposes, any dose of PCV13 given after six weeks of age, at least four weeks after the previous dose, and at least 30 days before the hospital admission date, was considered valid.

Laboratory methods

Microbiology included blood and pleural fluid cultures. Real-time polymerase chain reaction (RT-PCR) was performed as previously described.[25] Briefly, a panel of primers and probes for 14 pathogens *(S. pneumoniae, Neisseria meningitidis, Haemophilus influenzae, Escherichia coli, Klebsiella pneumoniae, Streptococcus agalactiae, Listeria monocytogenes, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Salmonella spp, Mycoplasma pneumoniae, Fusobacterium spp, and Adenovirus)* was used for routine diagnosis. Etiological diagnosis was made if RT-PCR and/or culture was positive in blood or pleural fluid samples. When RT-PCR was negative for all primers/probes included in the panel, amplification and sequencing of the 16S rRNA bacterial gene were performed. All samples positive for *S. pneumoniae* were serotyped by RT-PCR using 33 primer couples and probes. Pneumococcal serotypes were classified as PCV7 serotypes (4, 6B, 9V, 14, 18C, 19F, 23F), PCV13 serotypes (PCV7 plus 1, 3, 5, 6A, 7F, 19A), and non-PCV13 serotypes (not included in the PCV13). If no increase in fluorescent signal was observed after 40 cycles for any of the serotype-specific primer/probe sets, in spite of a positive result with both RT-PCR (lytA gene) and end-point PCR

(cpsA gene), the sample was reported as non-typeable. Viral studies were performed in a limited number of patients on a nasopharyngeal swab or on bronchoalveolar lavage fluid or on both, as previously described.[26]

Statistical Analysis

Results were summarized as medians and interquartile ranges (IQR) for continuous variables, and percentages for nominal variables. Continuous variables were compared by means of Mann–Whitney U test. Chi-square or Fisher's test were performed to evaluate differences between categorical variables. Chi-square for linear trend analysis was performed to determine significance in incidence trends. $p < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS (Version 26.0, SPSS, Inc., Chicago, IL, USA) and the freely available "openepi" package (https://www.openepi.com/Menu/OE_Menu.htm).

RESULTS

Demographics and clinical data

We identified 49 children with NP from our database. Six of them were excluded (congenital lung abnormalities n. 1, cystic fibrosis n. 1, primary ciliary dyskinesia n. 1, neurological compromise n. 1, haemophagocytic lymphohistiocytosis n. 1, pneumonia due to *M. tuberculosis* n. 1). Therefore, 43 children (18 males, 25 females) were considered.

The median age was 44 months (IQR 32-62). Most children (40/43, 93%) were previously healthy. Thirty-nine children had received antibiotic treatment before hospitalization. Thirty-two children had associated empyema or parapneumonic effusion. Two patients developed bronchopleural fistula. Two children suffered from haemolytic uremic syndrome, both in association with pneumococcal pneumonia. Median hospitalization length was 20 days (IQR 17-28). Nineteen children (44%) were admitted to a pediatric intensive care unit (PICU) for a median duration of 8 days (IQR 4-26), and eleven of them (58%) were intubated. Fifteen patients received pleural drainage alone; intrapleural urokinase or video-assisted thoracoscopic surgery were necessary in four and eight cases, respectively; one patient underwent lobectomy. Demographic characteristics, season of hospitalization, clinical features, etiologic agents and hospital course in the pre- or post-PCV13 period are shown in the Table 1. No differences in age, sex, season of hospitalization, comorbidity, clinical presentation, hospital course and etiologic agents were identified between pre- and post-PCV13 periods. The median age of children admitted to PICU (32 months, IQR 32-62) was lower than that of those who were not admitted to PICU (51 months, IQR 38-77) (p=0.002).

Necrotizing pneumonia-associated hospitalizations

When the rate of NP-associated hospitalizations of each study period was compared to the previous one, a significant difference was found between the early (1.5/1000 admissions/year) and the intermediate (0.35/1000 admissions/year) post-PCV13 period (p=0.001) (Figure 1). An increase in hospitalization rate was found between the intermediate and the late post-PCV13 period (0.94/1000 admissions/year). Chi-square for linear trend in the late post-PCV13 period was 2.14 (p= 0.13).

Bacterial species distribution

Overall, etiological diagnosis was achieved in 32/43 (74%) cases. *S. pneumonia*e was the most frequent pathogen identified (24/43, 56%), followed by *S. pyogenes* (5/43, 12%) and *S. aureus*

(3/43, 7%). Bacterial etiologies of NP during all study period, pre-PCV13 period and post-PCV13 periods are shown in the Table 2. A significant difference in the hospitalization rate of NP caused by *S. pneumonia*e was found between the early (0.86/1000 admissions/year) and the intermediate (zero/1000 admissions/year) post-PCV13 period (p=0.001). An increasing trend in the hospitalization rate was found between the intermediate and the late (0.58/1000 admissions/year) post-PCV13 period (p=0.06). There was no difference in the hospitalization rate for NP caused by *S. pyogenes* or *S. aureus* between pre- and post-PCV13 periods. There was no difference in the admission rate to PICU between different etiologic agents; however, all three children with *S. aureus* infection had to be admitted to PICU.

Vaccine coverage

A documented vaccination status was available in 39/43 (91%) patients. Vaccination against pneumococcus was ascertained in 37 children (86%); all of them had received at least two doses of vaccine; 27 patients had received PCV7 vaccination, and 10 had received PCV13 vaccination. Pneumococcal vaccine coverage in children during the different study periods is shown in the Table 3. All children with *S. pneumoniae* NP during the early post-PCV13 period had received PCV7 vaccination. In children who had received PCV13 vaccination, *S. pneumoniae* NP was present only in the late post-PCV13 period.

Microbiological findings

Cultures and RT-PCR analysis were obtained in 23 and 23 blood samples, and in 17 and 22 pleural fluid samples, respectively. Culture and RT-PCR analysis were positive in 5/23 (22%) and 13/23 (57%) blood samples, and in 5/17 (29%) and 21/22 (95%) pleural fluid samples, respectively. Overall, pleural fluid was more informative than blood in revealing etiology: 26/39 (67%) vs 18/46 (39%). RT-PCR analysis was 2.6 and 3.3 times more sensitive than culture in achieving etiological diagnosis in blood and pleural fluid samples, respectively. Viral coinfections were detected in 4 cases: influenza virus A H1N1 (n. 1), influenza B (n. 1), respiratory syncytial virus (n. 1), rhinovirus (n. 1).

***S. pneumoniae* serotyping**

The sample amount was not enough to perform serotyping in 5/24 patients with *S. pneumoniae* infection. Pneumococcal serotype-specific detection was available in 17/19 (89%) children. Serotype distribution in pre-PCV13 and post-PCV13 periods is shown in the Table 4. Among 13 pneumococcal isolates recovered in the post-PCV13 periods, 9 serotypes were

218 available, and they were mainly vaccine serotypes (serotypes 1 [n=2], 3 [n=4], 7F [n=1]).
219 Serotype 3 was found in 4 of 5 (80%) children who had received PCV13 immunization.

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DISCUSSION

To our knowledge, this is the first study reporting the epidemiology of NP in children before and after PCV13 vaccination. Our study shows that the hospitalization rate for NP in Italy significantly decreased a few years after PCV13 implementation. This finding is not surprising since all children who developed NP during the early post-PCV13 period had received PCV7 vaccination, mainly because they were born in the pre-PCV13 era. However, an increased trend in hospital admissions was found in the late post-PCV13 period, especially due to *S. pneumoniae* cases. Noteworthy, all children with NP in such period had received PCV13 vaccination.

Albeit a reduced incidence of complicated pneumonia in childhood has been observed in many studies following replacement of PCV7 with PCV13,[16-24] this finding has not been universal. Indeed, recent studies reported increasing cases of complicated pneumonia over the last few years. In a national surveillance study in Germany, the incidence of *S. pneumoniae* empyema significantly decreased from 3.5 per million children in 2010/11 to 1.5 in 2013/14, followed by a re-increase to 2.2 by 2016/17.[23] A retrospective study in Australia showed increasing rates of admissions and higher severity of empyema cases in children between 2011 and 2018.[27] In a population-based cohort study of invasive pneumococcal disease in Canada, rates of childhood empyema increased from 2000 to 2004 (4.0%), 2005 to 2009 (7.2%) and 2010 to 2014 (15.7%).[28] Only one study has reported the epidemiology of NP in childhood over the years. The annual average number of children hospitalized with NP in a tertiary medical center in Jerusalem from 2015 to 2017 (n=5.3) doubled compared to that from 2001 to 2014 (n=2.6).[29] However, the lack of information on the immunization status of patients does not allow inferring the effectiveness of pneumococcal vaccination.

The underlying causes of the increased trend in hospital admissions for complicated pneumonia over the last years are not fully understood. We can reasonably exclude a reduced primary care antibiotic prescription in our patients, since variations in this practice have not been reported in Italy. (https://www.ecdc.europa.eu/en/antimicrobial-consumption/database/country-overview). A national study in Italian children with empyema showed that the Red Queen hypothesis[30] - that is the increased incidence of non-PCV13 serotypes after PCV13 implementation - is hard to accept.[21] Host genetic[31] and microorganism factors such as a high bacterial load in the blood[32] may be responsible. Indeed, the higher

sensitivity of both RT-PCR (57%) and culture (22%) in blood samples of our patients compared to that in children with empyema (17% and 7%, respectively)[21] supports this hypothesis.

A recent review reported that *S. pneumonia*e was the most frequent etiologic agent (59%) of NP, mostly in the pre-PCV13 period, followed by *S. aureus* (23%), *Mycoplasma pneumonia*e (6%) and *S. pyogenes* (2.5%).[4] We found that *S. pneumoniae* was still the most common cause of NP (52%) in the post-PCV13 period. However, 10/19 (53%) children with NP caused by *S. pneumoniae* in the post-PCV13 period had not been vaccinated with PCV13. The hospitalization rate for NP associated with *S. pyogenes* and *S. aureus* did not differ between pre- and post-PCV13 periods. This finding is in contrast with the reported change in the bacterial etiology of childhood empyema after the implementation of PCV13.[19,23,33,34,35] Although we didn't find temporal changes in the bacterial etiology of NP, seven out of eight patients with *S. pyogenes* or *S. aureus* infection were admitted during the post-PCV13 period.

The change induced by pneumococcal vaccination in the prevalence of *S. pneumoniae* serotypes in invasive pneumococcal disease has been a matter of critical speculation.[15] In children with NP, non-vaccine serotypes have been most commonly implicated before and after the introduction of the PCV7. In Northern England, serotypes 1 and 3 were found in 7/13 (53%) patients with cavitatory lung disease associated with empyema.[36] In Texas, serotype 19A was an emerging cause (4/11, 36%) of NP and was associated with a more complicated course of illness.[37] In a series of 15 children with NP from Taiwan, serotypes 14 and 3 were found in 50% and 30% of cases, respectively.[7] Furthermore, serotypes 3 and 19A accounted for 80% of *S. pneumonia*e isolates in 41 children admitted with NP to a teaching hospital in France.[38]

Our study shows that pneumococcal strains recovered in the post-PCV13 period were mainly PCV13 vaccine serotypes (1, 3, 7F). Furthermore, all cases of PCV13 vaccine failure except one were due to serotype 3. This finding confirms that the efficacy of PCV13 against serotype 3 in invasive pneumococcal disease, especially complicated pneumonia,[39,40] is modest.[41] Serotype 3 has been reported to be associated with severe lung complications, such as pulmonary necrosis[42] and bronchopleural fistula.[43] Intrinsic properties may help serotype 3 evade and minimize immune responses.[44] In addition, higher levels of anti-capsular polysaccharide antibody concentration required to prevent infective complications may not be reached for some serotypes by the standard vaccination protocol.[45] Worthy of note, a large cohort study in UK demonstrated no impact of PCV13 vaccination on serotype 3 carriage or disease.[46]

Two of our 24 patients with pneumococcal NP complicated with haemolytic-uremic syndrome. Clinicians should be aware of this potential association, especially in young children.[20] Early recognition is indeed important to reduce morbidity and mortality.[47]

There are a number of limitations to our study. First, although a strict coding methodology was used to review the medical records, the retrospective design may have resulted in registration errors. Second, the relatively low number of patients may not allow unmasking ongoing changes in the bacterial etiology of NP. Third, viral studies were not conducted systematically. A high rate of viral coinfection has been reported in children with complicated pneumonia,[20] and virus-induced epithelial damage may be a predisposing factor for the bacteria-activated necrotizing process.[48] Although we cannot exclude differences in the rate of viral infections during the pre- and post-PCV13 periods, this is difficult to accept in the context of similar distribution of cases over seasons during the whole study period.

The strengths of our study include the involvement of two tertiary care pediatric hospitals that serve a stable population of about 500,000 children, the high pneumococcal vaccine coverage of the population, a detailed assessment of clinical characteristics of the patients, standardized microbiological analyses, and the 15-year period of the study.

In conclusion, our study showed a reduced incidence of hospitalizations for NP at two children's hospitals in Italy a few years after PCV13 implementation. An increased trend in hospital admissions was found in the late post-PCV13 period, especially due to *S. pneumoniae* cases. *S. pneumoniae* serotype 3 was the most frequent causal agent in both pre- and post-PCV13 periods. Although we didn't find ongoing changes in the bacterial etiology of NP, most patients with *S. pyogenes* or *S. aureus* infection were admitted during the post-PCV13 period. Future studies are needed to assess the epidemiology of NP in children who received PCV13 vaccination. Continued surveillance is also required to monitor pneumococcal serotype replacements and shifts in the coming years. Future pneumococcal vaccines should not only cover newly emerging serotypes, but also include a more effective component against serotype 3.

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CONFLICTS OF INTEREST

All the authors declare no competing interests.

AUTHORS' CONTRIBUTION

Conceived and designed the study: FMdB. Acquired and managed data: CR, GC, GR, SR. Performed statistical analysis: CR, SR. Searched literature: IC. Grafted the manuscript: FMdB, IC. Critical reviewing of the manuscript: CA, CR, SR. Revised and approved the final version of the text: All authors.

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