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Increase in *Streptococcus pneumoniae* serotype 3 associated parapneumonic pleural effusion/empyema after the introduction of PCV13 in Germany

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ABSTRACT

Introduction: Pediatric pneumococcal pneumonia complicated by parapneumonic pleural effusion/empyema (PPE/PE) remains a major concern despite general immunization with pneumococcal conjugate vaccines (PCVs).
Methods: In a nationwide pediatric hospital surveillance study in Germany we identified 584 children <18 years of age with bacteriologically confirmed PPE/PE from October 2010 to June 2018. *Streptococcus pneumoniae* was identified by culture and/or PCR of blood samples and/or pleural fluid and serotyped.
Results: S. pneumoniae was identified in 256 of 584 (43.8%) children by culture (n = 122) and/or PCR (n = 207). The following pneumococcal serotypes were detected in 114 children: serotype 3 (42.1%), 1 (25.4%), 7F (12.3%), 19A (7.9%), other PCV13 serotypes (4.4%) and non-PCV13 serotypes (7.9%). Between October 2010 and June 2014 serotype 1 (38.1%) and serotype 3 (25.4%) were most prevalent, whereas between July 2014 and June 2018 serotype 3 (62.7%) and non-PCV13 serotypes (15.7%) were dominant. Compared to children with other pneumococcal serotypes, children with serotype 3 associated PPE/PE were younger (median 3.2 years [IQR 2.1–4.3 years] vs. median 5.6 years [IQR 3.8–8.2 years]; $p < 0.001$) and more frequently admitted to intensive care (43 [89.6%] vs. 48 [73.8%]; $p = 0.04$). Seventy-six of 114 (66.7%) children with pneumococcal PPE/PE had been vaccinated with pneumococcal vaccines. Thirty-nine of 76 (51.3%) had received a vaccine covering the serotype detected. Thirty of these 39 breakthrough cases were age-appropriately vaccinated with PCV13 and considered vaccine failures, including 26 children with serotype 3, three children with serotype 19A and one child with serotype 1.
Conclusion: Following the introduction of PCV13 in general childhood vaccination we observed a strong emergence of serotype 3 associated PPE/PE in the German pediatric population, including a considerable number of younger children with serotype 3 vaccine breakthrough cases and failures. Future PCVs should not only cover newly emerging serotypes, but also include a more effective component against serotype 3.

1. Introduction

Community-acquired pneumonia (CAP) is still the leading cause of morbidity and mortality in young children beyond the neonatal period [1,2]. An annual rate of approximately 1000 pneumonia hospitalizations per 100,000 children <2 years has been estimated for the United States [3]. Up to 5% of patients admitted to hospital with pneumonia develop parapneumonic pleural effusions [4]; of

Abbreviations: PPE/PE, parapneumonic pleural effusion/empyema; PCV, pneumococcal conjugate vaccine; CAP, community-acquired pneumonia; IPD, invasive pneumococcal disease; SY, study year; BC, breakthrough case; VF, vaccine failure.

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these, 5–10% progress to complicated parapneumonic effusion/empyema (PPE/PE) [5]. *Streptococcus pneumoniae* is the major cause of pediatric PPE/PE besides other aerobic, gram positive cocci, such as *Streptococcus pyogenes* and *Staphylococcus aureus* [6–8].

Pneumococcal vaccines have been developed based on serotype-specific capsular antigens of *S. pneumoniae*. In 2001, a pneumococcal conjugate vaccine (PCV) targeting seven pneumococcal serotypes most frequently associated with invasive pneumococcal disease (IPD) was licensed in Germany (serotypes: 4, 6B, 9V, 14, 18C, 19F and 23F) [9,10]. In 2006, the German Standing Committee on Vaccination (STIKO) issued a recommendation for general pneumococcal vaccination of all children <2 years for the prevention of IPD in a 3 + 1 schedule at 2, 3, 4 and 11–14 months of age [11]. By 2009, a vaccination coverage of 81% had been reached in the pediatric population [12]. Since 2009, PCV7 has been successively replaced by higher-valent PCVs. PCV10 included serotypes 1, 5 and 7F in addition to the serotypes covered by PCV7, and PCV13 included the additional serotypes 1, 3, 5, 6A, 7F and 19A. By 2010–2012, PCV13 had reached a market share of 91% [13]. In 2015, the recommendation for infants in Germany was modified to a 2 + 1 schedule at 2, 4 and 11–14 months of age [14].

After the introduction of PCV7 in the USA in 2000, the incidence of hospital admissions due to CAP decreased from 1267 cases (1996–1998) to 852 cases (2005–2007) per 100,000 children <2 years of age [15]. Although the incidence of CAP complicated by PPE/PE was substantially lower, it doubled from 3.5 cases to 7.0 cases in 100,000 children <2 years of age within the same period [15]. The pneumococcal serotypes 1, 3, 7F and 19A, which were suspected to have a higher potential to invade the pleural space, were not included in PCV7 and were increasingly detected in children with PPE/PE after PCV7 introduction [16–19]. The decrease in CAP incidence paired with a rise in PPE/PE incidence following PCV7 introduction therefore suggested a vaccine-induced serotype replacement. In line with this hypothesis, several countries reported decreasing incidence rates of pediatric PPE/PE shortly after the introduction of PCV13 (including serotypes 1, 3, 7F and 19A) [20–23]. Similarly, we observed an initial decrease of PPE/PE incidence in Germany following PCV13 introduction in 2009/10, which was, however, followed by an increase in PPE/PE incidence from 2013/14 [7].

The increase of PPE/PE may be due either to a lower effectiveness against a specific serotype included in the vaccine or to the replacement of vaccine serotypes with non-vaccine serotypes in vaccinated children. Interestingly, an increase of PPE/PE has also been observed in countries without PCV-immunization programmes (e.g., Israel), suggesting secular trends in the distribution and virulence of different pneumococcal serotypes [24,25]. In this study we investigated *S. pneumoniae* associated PPE/PE in PCV vaccinated children on a serotype specific level in the context of changing pneumococcal vaccination in Germany.

2. Methods

The study population included children <18 years of age admitted to hospital for pneumonia-associated PPE/PE either requiring drainage or persisting >7 days. Children with PPE/PE were identified by prospective surveillance in all 472 pediatric hospitals/departments in Germany from October 2010 to June 2018. A study year (SY) covered the period from July 1 in one year to June 30 in the following year, except for SY1 (October 2010 to June 2011) [7,26]. Children with pneumococcal PPE/PE and documented pneumococcal vaccination status were included in the analysis.

Bacteria and/or bacterial DNA identified in blood samples and/or pleural fluid by culture and/or PCR were considered causative for PPE/PE. In addition to standard local hospital procedures, broad-spectrum 16S rRNA gene sequencing of pleural fluid at the Institute of Hygiene and Microbiology, University of Würzburg was offered to all reporting clinicians. When pleural fluid was forwarded to the University of Würzburg or the national reference center for pneumococci in Aachen, pneumococcal serotyping was performed using multiplex polymerase chain reaction and subsequent gel electrophoresis according to published methods [27,28]. Only written documentation of pneumococcal vaccination status with information on the trade name of the vaccine and the date of vaccination for each dose was accepted for analysis. All pneumococcal vaccine doses given at least 14 days prior to hospital admission for PPE/PE were considered relevant. Based on the successive introduction of PCVs in the German vaccination schedule, birth cohorts were classified as pre-PCV7 ($\leq$2005), PCV7 (2006–2008) or post-PCV7 ($\geq$2009).

Children vaccinated with pneumococcal vaccines covering the pneumococcal serotype detected were considered vaccine breakthrough cases (BCs) [29]. In line with the recommendations of the Council for the International Organizations of Medical Sciences/WHO Working Group on Vaccine Pharmacovigilance, BCs with full and age-appropriate vaccination were considered vaccine failures (VFs) [30–32]. The German pneumococcal vaccination recommendation was modified from four doses (3 + 1) to three doses (2 + 1) in August 2015 [14]. On the assumption that the 2 + 1 schedule provided full protection against vaccine-type diseases also for children born before August 2015, we defined age-appropriate vaccination for all children according to the current recommendations of the German Standing Committee on Vaccination, i.e. as immunization with at least two doses of PCV at 2–4 months of age followed by a booster dose at 11–14 months of age [11,14].

Continuous data is reported as median (IQR, 25th percentile to 75th percentile). P-values were calculated using the Mann-Whitney *U* test. Nominal and ordinal data are reported as numbers of children (percent). P-values were calculated using Fisher's exact test or the asymptotic Chi-Squared test, as appropriate. Clinical data are reported stratified by evidence of serotype 3. Explorative investigation of linear trends in count data across study years was performed using loglinear Poisson regression models. Statistical analyses were carried out using IBM SPSS Statistics Version 25 (IBM Corporation, One New Orchard Road, Armonk, New York, USA).

The local Ethics Committee of the Medical Faculty and the Data Protection Office of the University Hospital of Würzburg approved the study protocol and amendments (reference number 140/10).

3. Results

A total of 1724 children with pneumonia complicated by PPE/PE were enrolled in the study. In 584 of 1724 (33.9%) children a bacterial pathogen was identified. *S. pneumoniae* was detected in 256 of 584 (43.8%) children by culture (n = 122) and/or PCR (n = 207). Of these 256 children, 168 (65.6%) had been vaccinated with pneumococcal vaccines, 53 (20.7%) had not been vaccinated and for 35 (13.7%) the pneumococcal vaccination status was unknown. Serotyping was performed for 114 of 256 (44.5%) isolates from children with pneumococcal PPE/PE. Of these 114 children, 76 (66.7%) had been vaccinated with pneumococcal vaccines, 8 (7.0%) children had been vaccinated but the vaccine tradename was unknown, 18 (15.8%) children had not been vaccinated and for 12 (10.5%) children the pneumococcal vaccination status was unknown.

In 114 children with pneumococcal PPE/PE and serotyping, the following serotypes were identified: serotype 3 (48, 42.1%), 1 (29,

25.4%), 7F (14, 12.3%), 19A (9, 7.9%), 8 (4, 3.5%) and 9 V, 11A, 12F, 18C, 19F, 22F, 23F, 35B, 35F and serotypes 1 + 3 in one child each (1, 0.9%, respectively). Non-PCV13 serotypes were identified in 9 children: serotype 8 (4 children), serotype 11A, 12F, 22F, 35B and 35F (one child each). As shown in Table 1, the proportion of serotype 1 (38.1% vs. 9.8%; p < 0.01) and 7F (19.0% vs. 3.9%; p = 0.01) among all detected pneumococcal serotypes was higher between October 2010 and June 2014 (SYs 1–4) than in the period from July 2014 to June 2018 (SYs 5–8). In contrast, the proportion of serotype 3 (25.4% vs. 62.7%; p < 0.001) and non-PCV13 serotypes (1.6% vs. 15.7%; p < 0.01) was lower in the early study period (SYs 1–4 vs. SYs 5–8). We observed decreasing seasonal trends for serotype 1 (p for trend < 0.01) and 7F (p for trend = 0.04), and an increasing seasonal trend for serotype 3 (p for trend = 0.02; adjusted for age and gender).

Compared to children with other pneumococcal serotypes, children with serotype 3 associated PPE/PE were more often female (31 [64.6%] vs. 24 [36.9%]; p < 0.01), younger (median 3.2 years [IQR 2.1–4.3 years] vs. median 5.6 years [IQR 3.8–8.2 years]; p < 0.001) and more frequently born in the post-PCV7 period (39 [81.3%] vs. 15 [23.1%]; p < 0.001) (Table 2). Furthermore, children with serotype 3 associated PPE/PE were hospitalized longer (median 22.0 days [IQR 18.0–29.0 days] vs. median 17.5 days [IQR 14.0–24.5 days]; p = 0.02) and more frequently admitted to intensive care units (43 [89.6%] vs. 48 [73.8%]; p = 0.04). There were no differences regarding preexisting comorbid conditions and clinical outcome.

For 76 of 114 (66.6%) children with pneumococcal serotyping, the pneumococcal vaccine tradename and vaccination date were documented; 39 of these 76 (51.3%) children had received pneumococcal vaccines potentially covering the detected pneumococcal serotype (Supplemental Table 1). Among these, children with age-appropriate pneumococcal vaccination were considered VFs (n = 34), whereas children with non-age-appropriate vaccination were considered BCs only (n = 5). Only 2 of 39 (5.1%) BCs had a preexisting immune disorder, potentially predisposing them for decreased vaccine effectiveness. As shown in Table 3, 26 of 48 (54.2%) children with serotype 3 associated PPE/PE had been age-appropriately vaccinated with PCV13 and were considered VFs. Further, 2 of 48 (4.2%) children with serotype 3 had been non-age-appropriately vaccinated with PCV13 and were considered BCs only. In addition, 3 of 9 (33.3%) children with serotype 19A and one of 29 (3.4%) children with serotype 1 had been age-appropriately vaccinated with PCV13 and were considered VFs. Breakthrough disease cases or VFs due to serotype 7F were not observed. In 37 of 76 (48.7%) vaccinated children, serotypes not covered by the administered vaccine were detected. They were considered non-BCs. Serotype 3 was overrepresented among the subgroup of children considered as VFs compared to the group of all children with a pneumococcal serotype detected (26 of 34 [76.5%] vs. 48 of 114 [42.1%]).

Corresponding to the increase in children with PPE/PE vaccinated with PCV13, the number and proportion of children with breakthrough disease increased in the eight-year study period (Fig. 1). Whereas no children considered as BCs were detected in SY1, we observed an increase in the proportion of breakthrough cases to 6 of 7 (85.7%) children in SY8 (p for trend <0.01; adjusted for age and gender). The incidence of hospital admissions due to pneumococcal PPE/PE decreased from 3.4 (95%CI: 2.5–4.6) cases per one million children in SY1 to 1.5 (95%CI: 0.9–2.4) in SY4 and rose again to 2.9 (95%CI: 2.1–4.0) in SY7 and 2.1 (95%CI: 1.4–3.0) in SY8 (Supplemental Table 2). A similar pattern was observed for the incidence of hospital admission due to all-cause PPE/PE: 16.2 (95%CI: 14.1–18.5) cases per one million children in SY1; 13.7 (95%CI: 11.8–15.8) in SY4; 18.2 (95%CI: 16.0–20.6) in SY7 and 17.1 (95%CI: 14.9–19.4) in SY8. The incidence of serotype 3 associated PPE/PE fluctuated between 0.08 (95%CI: 0.00–0.43) and 1.19 (95%CI: 0.68–1.93) cases per one million children, without a clear decreasing trend.

4. Discussion

There is substantial evidence that PCVs prevent invasive pneumococcal disease in children and adults. Several countries have reported falling incidence rates of CAP [3,33–35] and IPD [36–38] after the introduction of PCVs. Some countries have also reported decreasing rates of PPE/PE [21,22,39], whereas this was not the case in all countries [7]. In our national surveillance study, we studied changes in the incidence and serotype distribution of pediatric pneumococcal PPE/PE following the introduction of general pneumococcal vaccination with PCV7, PCV10 and PCV13. Between October 2010 and June 2018, the pneumococcal serotypes 1, 3, 7F, 19A and non-PCV13 serotypes (serotypes 8, 11A, 12F, 22F, 35B and 35F) accounted for 95.2% of all serotypes detected in children <18 years of age with pneumococcal PPE/PE in Germany. Following the introduction of general PCV13 pneumococcal vaccination for infants in 2009/2010, the incidence of pneumococcal and all-cause PPE/PE decreased in the years until 2013/14, followed by a subsequent increase [7]. Although there was a clear decrease in children with pneumococcal PPE/PE caused by serotypes 1 and 7F, the number of children with non-PCV13 serotypes and in particular the number of children with serotype 3 associated PPE/PE showed a strong increase. A recent report including children with

Table 1
Pneumococcal serotypes of 256 children <18 years in Germany hospitalized between October 2010 and June 2018 with pneumococcal parapneumonic pleural effusion/empyema (PPE/PE), stratified by **early period (October 2010 to June 2014)** and **late period (July 2014 to June 2018)**.

	Early period SY1 - SY4 139 (1 0 0)	Late period SY5 - SY8 117 (1 0 0)	p value	Trend analysis*	p value for trend
Not serotyped	76 (54.7)	66 (56.4)	0.78	not performed	
Any serotype	63 (45.3)	51 (43.6)	0.78	not performed	
1	24 (38.1)	5 (9.8)	<0.01	0.73 (95%CI: 0.59–0.88)	<0.01
7F	12 (19.0)	2 (3.9)	0.01	0.73 (95%CI: 0.55–0.98)	0.04
19A	7 (11.1)	2 (3.9)	0.16	0.82 (95%CI: 0.61–1.10)	0.19
3	16 (25.4)	32 (62.7)	<0.001	1.16 (95%CI: 1.02–1.32)	0.02
Other PCV13	3 (4.8)	2 (3.9)	0.83	convergence criteria not satisfied	
Non-PCV13	1 (1.6)	8 (15.7)	<0.01	convergence criteria not satisfied	

Data reported: n (percent) and p-values for Chi-Squared test.
Percentages of individual serotypes refer to all children with serotype information.
Other PCV13 serotypes: 9V, 1 + 3, 18C, 23F and 19F.
Non-PCV13 serotypes: serotype 8 (4 children), 11A, 12F, 22F, 35B and 35F (one child each).
* Trend analysis was done by loglinear Poisson regression and adjusted for age and gender. Estimates >1 indicated an increasing trend.

Table 2
Demography and **clinical data** of 113 children <18 years in Germany hospitalized between October 2010 and June 2018 with pneumococcal parapneumonic pleural effusion/empyema (PPE/PE) and with available data on serotype, stratified by **serotype 3 and non-serotype 3**. One child with evidence of serotype 1 and 3 was excluded.

		Serotype 3 48 (100)	Non-Serotype 3[a] 65 (100)	p value
Sex	Female	31 (64.6)	24 (36.9)	<0.01
Age, years	Median (IQR)	3.2 (2.1; 4.3)	5.6 (3.8; 8.2)	<0.001
Birth cohort	≤ 2005 (pre-PCV7)	1 (2.1)	23 (35.4)	
	2006–2008 (PCV7)	8 (16.7)	27 (41.5)	
	≥ 2009 (post-PCV7)	39 (81.3)	15 (23.1)	<0.001
Preexisting condition	Any	10 (20.8)	21 (32.3)	0.18
	Immunodeficiency	0 (0.0)	3[b] (4.6)	0.26
	Premature birth	2 (4.2)	5 (7.7)	0.70
Disease severity	Hospitalization duration[c], days	22.0 (18.0; 29.0)	17.5 (14.0; 24.5)	0.02
	ICU admittance	43 (89.6)	48 (73.8)	0.04
	ICU duration[d], days	9.5 (7.0; 15.0)	7.0 (3.0; 14.0)	0.20
Outcome	Recovered with sequelae	15 (31.3)	14 (21.5)	0.24
	Fatality	0 (0.0)	0 (0.0)	–
Time since last vaccination[e], months	Last vaccination to admission	21.4 (8.9; 31.7)	40.2 (28.6; 61.4)	<0.001

Data reported: n (percent) or median (IQR), p-values for Fisher's exact test, Chi-Squared test or Mann-Whitney *U* test, as appropriate.
PCV, pneumococcal conjugate vaccine; **ICU**, intensive care unit.
[a] Non-Serotype 3 serotypes: serotype 1 (29, 44.6%), 7F (14, 21.5%), 19A (9, 13.8%), 8 (4, 6.2%), and 9V, 11A, 18C, 12F, 19F, 22F, 23F, 35B and 35F (1 child each, 1.5%).
[b] One child had an antibody deficiency syndrome (Bruton Agammaglobulinemia), one child an inactivation of interleukin 6, and one child was considered immune deficient due to premature birth with neurological sequelae (callosal agenesis, West-Syndrome).
[c] Duration of hospitalization was available for 111 of 113 children.
[d] Duration of intensive care was available for 82 of 91 children who were admitted to an intensive care unit.
[e] Time since last vaccination was available for 75 children with pneumococcal serotyping and documentation of vaccination status.

Table 3
Pneumococcal serotypes of 256 children <18 years in Germany hospitalized between October 2010 and June 2018 with pneumococcal parapneumonic pleural effusion/empyema (PPE/PE), stratified by **pneumococcal vaccination status**.

Pneumococcal serotype	All	AP PCV7	n-AP PCV7	AP PCV13	n-AP PCV13	Other Vaccination[d]	Vaccination unknown	Not vaccinated
1	29 (100)	10 (34.5)	1 (3.4)	1 (3.4)	0 (0.0)	1 (3.4)	5 (17.2)	11 (37.9)
7F	14 (100)	5 (35.7)	1 (7.1)	0 (0.0)	0 (0.0)	4 (28.6)	1 (7.1)	3 (21.4)
19A	9 (100)	3 (33.3)	1 (11.1)	3 (33.3)	0 (0.0)	1 (11.1)	1 (11.1)	0 (0.0)
3	48 (100)	4 (8.3)	0 (0.0)	26[c] (54.2)	2 (4.2)	8 (16.7)	5 (10.4)	3 (6.3)
Other PCV13 [a]	5 (100)	2 (40.0)	1 (20.0)	0 (0.0)	1 (20.0)	1 (20.0)	0 (0.0)	0 (0.0)
Non-PCV13 [b]	9 (100)	0 (0.0)	0 (0.0)	3 (33.3)	2 (22.2)	3 (33.3)	0 (0.0)	1 (11.1)
Not serotyped	142 (100)	10 (7.0)	0 (0.0)	30 (21.1)	14 (9.9)	30 (21.1)	23 (16.2)	35 (24.6)
All	256 (100)	34 (13.3)	4 (1.6)	63 (24.6)	19 (7.4)	48 (18.8)	35 (13.7)	53 (20.7)

Data reported: n (percent).
AP, age-appropriate pneumococcal vaccination; **n-AP**, non-age-appropriate pneumococcal vaccination.
[a] Other PCV13 serotypes: serotype 9V, 1 + 3, 18C, 23F and 19F (one child each).
[b] Non-PCV13 serotypes: serotype 8 (4 children), 11A, 12F, 22F, 35B and 35F (one child each).
[c] Three of 26 (11.5%) children defined as AP PCV13 with evidence of serotype 3 had been vaccinated according to a 2 + 1 schedule although they were born during the period of the 3 + 1 schedule recommendation.
[d] Other vaccination: PCV10 (12 children), pneumococcal polysaccharide vaccine (2 children), mixed schedule (11 children) and tradename unknown (23 children).

hospital admission due to IPD in Germany correspondingly showed a decreasing trend in the incidence of non-meningitis IPD after PCV13 introduction until 2012/13, followed by a subsequent rise in the incidence rates [40].

After the introduction of PCV7 an increase in serotype 3 IPD incidence was observed in children <18 years of age in the USA (incidence rate ratio = 2.45; 95%CI: 0.63–9.45) [41]. A study from Norway reported an increasing proportion of serotype 3 IPD isolates following the introduction of PCVs in children <5 years of age (pre-PCV7: 5 of 208 [2.4%] vs. PCV7: 13 of 260 [5.0%] vs. PCV13: 3 of 47 [6.4%] [42]. Following PCV13 introduction, other studies of pediatric IPD cases in the USA [43], Denmark [44] and Canada [36] showed moderate non-significant reductions of serotype 3 disease; whereas other PCV13 serotypes, in particular serotypes 1 and 7F, decreased substantially. One study based on a small number of children with culture-based evidence of *S. pneumoniae* in the USA showed a substantial (68%) reduction of serotype 3 IPD in the PCV13 period compared to the PCV7 period [45]. In another study from the USA, the incidence of hospital admissions due to CAP for *S. pneumoniae* serotype 3 showed a moderate, non-significant decrease following PCV13 introduction (7.3 [95% CI: 5.0–10.4] cases vs. 4.5 [95% CI: 2.7–6.9] cases per 100,000 admissions; −38%, p = 0.08) [35].

In accordance with our findings, *S. pneumoniae* serotype 3 was the most frequently detected serotype in children with pneumococcal PPE/PE in the PCV13 period in Portugal (40 of 109 [36.7%]) [19]. The same study identified 19 of 22 (86.4%) children with pneumococcal PPE/PE and age-appropriate PCV13 vaccination as vaccine failures; 17 were due to serotype 3 and one each due to serotype 1 and 14 [19]. Similarly, serotype 3 associated PPE/PE did not show a decrease despite increasing PCV13 vaccination coverage in Spain [46]. In Italy, the incidence rates of serotype 3 associated PPE/PE decreased by 31.5% following PCV13 vaccination, but serotype 3 remained the most frequent serotype in vaccinated children with pneumococcal PPE/PE (13 of 15 [86.6%]) [39].

In comparable analyses, children hospitalized due to PPE/PE were of a median age of 5 years [47]. Interestingly, in our cohort serotype 3 cases were clearly younger than other pneumococcal serotype cases (median 3.2 years [IQR 2.1–4.3] vs. median 5.6 years [IQR 3.8–8.2]; p < 0.001). The younger age of children with serotype 3 PPE/PE compared to children with PPE/PE due to other serotypes may indicate a shorter duration of vaccine induced

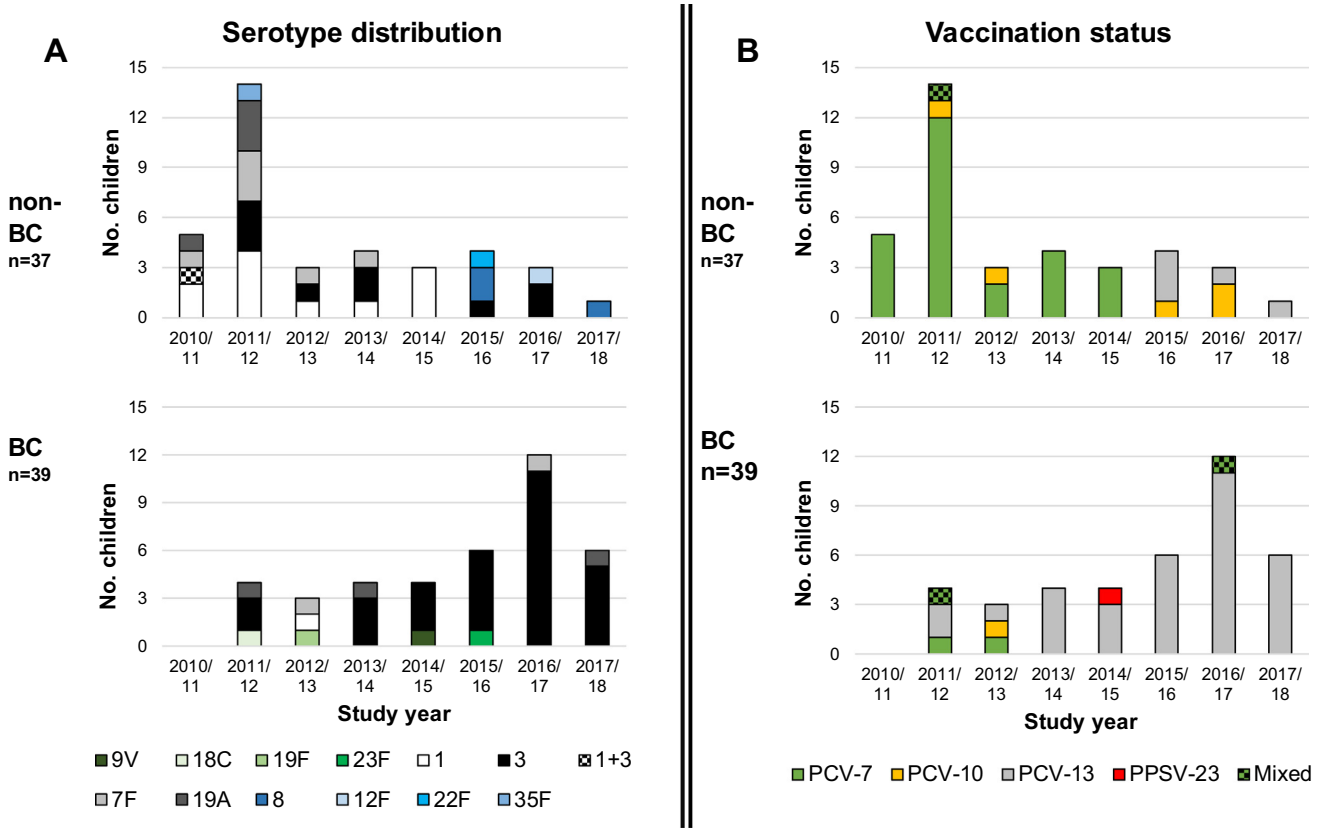

Fig. 1. Pneumococcal **serotype distribution [A]** and **vaccination status [B]** in children considered non-breakthrough case/breakthrough case (non-BC/BC). **Seasonal trend** of **pneumococcal serotype distribution [A]** and **pneumococcal vaccination status [B]** in 76 children <18 years in Germany hospitalized between Oct. 2010 and June 2018 with parapneumonic pleural effusion/empyema (PPE/PE) due to *S. pneumoniae* and with available data on pneumococcal serotype and pneumococcal vaccination status, stratified by **non-breakthrough cases (non-BC)** and **breakthrough cases**. In 2010/11 no children were considered to be BC. Vaccination status and vaccine serotypes for PCV7 are shown in green, PCV10 in gold, PCV13 in black/white/grey, 23-valent pneumococcal polysaccharide vaccine (PPSV-23) in red, mixed vaccines in checkered pattern and non-PCV13 in blue. Each study year started in July and ended in June, except for the first year (2010/11), which started in October.

protection against serotype 3 in comparison to other serotypes. The younger age may be one reason for the longer hospitalization duration and the higher proportion of admittance to intensive care units. Although an association of serotype 3 with younger age at hospital admission has not yet been shown [48–51], serotype 3 has been reported to be associated with a more severe and complicated course of disease. Bender et al. reported an association of serotype 3 with an increased risk for pneumococcal necrotizing pneumonia and radiographic evidence of lung necrosis [48]. Shen et al. reported serotype 3 as having the highest potential to cause pulmonary complications and being associated with a higher number of febrile days and a longer duration of hospitalization in children with IPD [49]. Asner et al. reported serotype 3 as being associated with higher rates of intensive care admission and longer duration of hospitalization in children with pneumococcal sepsis [50]. A systematic review and meta-analysis of children with pneumococcal pneumonia reported increased mortality for serotype 3 in comparison to serotype 14 [51]. It has been shown in several studies that, compared to other pneumococcal serotypes, *S. pneumoniae* serotype 3 has a higher potential to invade the pleural space and to necessitate a higher level of medical care [19].

Regarding vaccine immunogenicity, several studies have shown an attenuated ability of PCV13 to induce protective antibody titers for serotype 3 compared to other PCV13 serotypes [52–54]. Nevertheless a German study reported a 74% (2–93%) effectiveness of PCV 13 against serotype 3 associated IPD [55], and a recent systematic review and meta-analysis including four observational studies and a total of 146 pediatric serotype 3 IPD cases estimated a vaccine effectiveness of 63.5% for PCV13 against IPD due to serotype 3 [56]. Following discontinuation of PCV7 and the licensing and market introduction of PCV13 in late 2009, PCV13 has increasingly replaced vaccination with PCV7 in infants and children. In 2008/09, vaccination coverage in Germany was estimated at 81.2% and PCV13 accounted for 91% of all pneumococcal vaccinations in infants and children [12,13]. Starting in the study year 2014/15, we observed an increase in the number of PPE/PE in vaccinated children with breakthrough disease as well as VFs in previously healthy children mostly associated with *S. pneumoniae* serotype 3 disease. Preexisting immune disorders and premature birth were excluded as explanations for these observations. In support of our findings, serotype 3 and, to a lesser degree, serotype 19A were also detected in children with pneumococcal PPE/PE despite PCV13 vaccination in comparable analyses in Portugal, Spain, Italy and Greece [19,31,39,46,57]. Case reports and case series of pneumococcal disease due to serotype 3 in children vaccinated with PCV13 were first published for hemolytic uremic syndrome in 2013 [58] and PPE/PE in 2014 [57,59]. VFs with respect to serotypes 3 and 19A were also observed in children with IPD, however considerably less frequently than for PPE/PE [30,60].

The reason for the lower effectiveness of PCV13 against serotype 3 associated PPE/PE in contrast to serotype 3 associated IPD is unclear. It is known that nasopharyngeal carriage of pneumococci is related to the development of invasive and non-invasive pneumococcal disease [61]. An Israel-Palestinian surveillance study showed a significant increase of non-PCV13 serotypes and a non-significant increasing trend of serotype 3 in nasopharyngeal carriage following PCV13 implementation [62]. Correspondingly, a randomized controlled trial comparing children vaccinated with

PCV7 and PCV13 showed no significant difference in nasopharyngeal carriage of serotype 3 [63]. Choi et al. argued that the release of capsular polysaccharides during the growth of serotype 3 may interfere with antibody-mediated immune responses [64]. Lower vaccine-induced serotype 3 antibody concentrations in the pleural space or nasopharynx, compared to blood samples, may explain the lower effectiveness of PCV13 against serotype 3 associated pneumococcal PPE/PE in comparison with the proven vaccine effectiveness of PCV13 against serotype associated 3 IPD.

Our study is subject to various limitations. As we enrolled children <18 years of age with pneumonia complicated by PPE/PE lasting >7 days or requiring pleural drainage, milder forms of pediatric PPE/PE-complicated pneumonia were underrepresented in our study. Since participation in the surveillance program was voluntary and did not include financial compensation, case identification may have been incomplete, resulting in an underestimation of incidence rates. Recall bias may have resulted in an overrepresentation of severe cases of disease. Standard microbiological procedures for pathogen detection may have varied to a certain degree in local laboratories. To increase the sensitivity of bacterial detection, broad-spectrum 16S rRNA gene sequencing of pleural fluid and subsequent molecular serotyping was offered free-of-charge to all reporting clinicians. Since samples with *S. pneumoniae* detected in local clinical microbiological laboratories by either culture or PCR were not always forwarded to the German national reference center for further serotyping, a pneumococcal serotype was available only for about half of all *S. pneumoniae* associated PPE/PE cases. In addition, vaccination status was not universally available but could be identified for >80% of included children.

The strength of our analysis is that we were able to include a considerable number of children with PPE/PE through an active nationwide and well-established surveillance system in Germany. The eight-year observation period included the transition from PCV7 to early PCV13 and late PCV13, thus enabling us to study serotype epidemiology and vaccine BCs in PPE/PE across different periods of pneumococcal vaccine use in Germany.

5. Conclusion

Following the introduction of PCV13 in Germany, *Streptococcus pneumoniae* serotype 3 became the most frequently detected serotype besides non-PCV13 serotypes in children with pneumonia complicated by PPE/PE. *S. pneumoniae* serotype 3 associated PPE/PE was increasingly observed in children vaccinated with PCV13 and was associated with a more severe course of disease in a growing number of children at a younger age compared to PPE/PE caused by other serotypes. Continued surveillance is necessary to monitor ongoing serotype replacements and shifts in PPE/PE. Future pneumococcal conjugate vaccines should not only cover newly emerging pneumococcal serotypes, but also improve protection against serotype 3.

Authors' contributions

DG, AS and JGL had full access to the study data and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: DG, AS, DK, CS, RvK, MAR, MvdL, JGL. Acquisition, analysis, or interpretation of data: DG, AS, DK, CS, RvK, MAR, MvdL, JGL. Drafting of the manuscript: DG, AS, JGL. Critical revision of the manuscript for important intellectual content: DG, AS, DK, CS, RvK, MAR, MvdL, JGL. Statistical analysis: DG, AS. Funding obtained by AS, JGL. Administrative, technical, or material support: DG, DK, CS, MvdL. Study supervision: AS, JGL. All authors approved the final version for submission. All authors attest they meet the ICMJE criteria for authorship.

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Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [**DG** included elements of this publication in his master's thesis. **AS** received research grants, conference speaker's fees, fees for participation in advisory board meetings or travelling grants from Pfizer and GSK. **DK, CS** and **RvK** declare no conflicts of interest relevant to this article. **MAR** received research grants and speaker's fees from Pfizer, GSK, AbbVie, SPMSD, and Novartis Vaccines. **MvdL** received research funding from Pfizer, is a member of advisory boards for Pfizer and MSD and received speaker's fees and funding for congress participation from Pfizer and MSD. **JGL** received research grants, speaker's fees, and fees for participation in advisory board meetings from Pfizer Pharma GmbH, Germany, GlaxoSmithKline GmbH & Co. KG, Germany (GSK), SPMSD and MSD.].

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Appendix A. Supplementary material

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