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SEROTYPES ASSOCIATED WITH THE DEVELOPMENT OF PNEUMOCOCCAL PARA-PNEUMONIC EFFUSION IN ADULTS

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Abstract

Objectives

Serotypes 1, 3, 7F and 19A are implicated in childhood pneumococcal para-pneumonic effusion (PPE). It is not known whether the same is true for adult PPE.

Methods

A prospective cohort study was conducted over a two-year period. Consecutive adults admitted with community-acquired pneumonia (CAP) were studied. Pneumococcal serotype was identified from urine samples using a multiplex immunoassay.

Results

Of 920 patients recruited, 366 patients had pneumococcal CAP; 100 of these had PPE with a serotype determined in 73. Factors associated with PPE were age, Pneumonia Severity Index and serotype. Serotypes most associated with PPE were 1 (n=18/40, 45%), 19A (n=9/20, 45%), and 3 (n=8/20, 40%). Serotypes common in childhood PPE were independently associated with adult PPE (adjusted odds ratio (OR) 2.3; p=0.003). Serotypes not included in the 7-valent conjugate vaccine were more likely to be associated with PPE (OR 2.1; p=0.024) compared to those in the vaccine. Serotypes included in PCV-13 were as likely to be associated with PPE as those that are not (OR 0.8; p=0.301).

Conclusion

Serotypes 1, 3, 7F and 19A are independently associated with adult PPE, a similar finding to childhood PPE. Serotype replacement following pneumococcal vaccine implementation may influence the spectrum of clinical disease.

Introduction

Para-pneumonic effusions (PPEs) complicate community-acquired pneumonia (CAP) in adults in at least 10% of cases,[1-3] and are associated with poorer outcome.[4] There is substantial variation in the incidence of PPE by causative organism, with the incidence lower in *Mycoplasma pneumoniae* and viral infection,[5] but higher when the cause is *Streptococcus pneumoniae.*[6] The commonest cause of CAP, *S. pneumoniae,*[7, 8] may be divided into over 90 different serotypes,[9] many of which show distinctly different clinical features in both adults and children. Serotypes 1, 3, 19A and 7F have been particularly associated with development of pneumococcal PPE and empyema in children,[10-12] but no such association has been reported to date in adults. Although no risk factors have been specifically identified for the development of pneumococcal PPE, factors associated with the development of complicated pneumococcal CAP (defined as multi-lobar CAP, PPE or empyema) include chronic liver disease, high admission C-reactive protein levels, and creatinine level, with COPD a negative risk factor.[13] Risk factors for all-cause complicated PPE include age ≥60 years, alcoholism, pleuritic chest pain, tachycardia, leucocytosis, low albumin, low plasma sodium, and high platelet count. [14, 15]

Seven-, ten-, and thirteen-valent pneumococcal conjugate vaccines have been licensed in recent years for use in preventing pneumococcal disease in children (with nine- and eleven-valent vaccines trialled and not licensed, and a fifteen-valent vaccine under development). The seven-valent conjugate vaccine (PCV-7) was added to UK childhood immunisation schedules in September 2006, and was replaced by a thirteen-valent vaccine (PCV-13) in April 2010. Following the introduction of the conjugate vaccines to infant immunisation schedules a shift was observed in the serotypes seen in adult invasive pneumococcal disease towards non-vaccine type serotypes.[16, 17] Some studies have reported a relative rise in the incidence of all-cause and pneumococcal PPE in children following the introduction of PCV-7,[11, 18-20] as the serotypes commonly associated with pneumococcal PPE are not included in PCV-7, although in the UK a fall in paediatric empyema hospitalisations was observed.[21] It is too early to say whether there has been a subsequent decrease in the incidence of pneumococcal PPE in children following the introduction of PCV-13 (which includes serotypes 1, 3, 5, 6A, 7F and 19A) due to continued serotype replacement with non-PCV-13 serotypes. Monitoring of pneumococcal serotypes and their associated clinical patterns of disease is therefore important and has not been performed for pneumococcal PPE in adults to date.

The aims of this paper were to a) describe the serotype distribution of pneumococcal PPE in adult non-invasive CAP, and b) to ascertain whether serotypes prevalent in childhood PPE are also implicated in adult PPE, independent of potential confounding variables.

Methods

Study design

The study design and recruitment methods are described elsewhere, and participants in this study were drawn from an earlier publication.[22] This study is novel in that it presents a detailed analysis of the association between the different pneumococcal serotypes previously described and the occurrence of simple and complicated PPEs. Briefly, consecutive adult patients (aged ≥16 years) admitted between September 2008 and September 2010 with CAP to two large UK teaching hospitals were prospectively recruited as part of an observational cohort study. Patients were included if they had at least one acute symptom consistent with a lower respiratory tract infection (breathlessness, cough, sputum or fever), had new infiltrates on chest radiograph, and were treated by the admitting team for CAP. Patients were excluded if they had been admitted to hospital in the preceding ten days, had tuberculosis, or had post-obstructive pneumonia due to lung cancer.

Urine samples were obtained from each patient at the start of the admission episode These were tested using the Binax NOW® immunochromatographic test kit (Alere Ltd, Stockport, UK) and tested in batches by a multiplexed serotype-specific pneumococcal immunoassay using xMAP beads (Bio-Plex®, Bio-Rad, Hercules, USA). The assay detects fourteen pneumococcal serotypes (1, 3, 4, 5, 6A/C, 6B, 7F/A, 8, 9V, 14, 18, 19A, 19F and 23F) with a sensitivity of 79% and specificity of 99%.[23] Serotype was also determined in bacteraemic pneumococcal patients by means of slide agglutination with latex pool sera and standard group and factor sera (Statens Serum Institut, Copenhagen, Denmark) at the RSIL, HPA Microbiology Services Division: Colindale, London. Routine blood, pleural fluid, and sputum samples for bacteriology culture were collected by admitting teams according to local CAP guidelines and processed in the Nottingham University Hospitals Department of Clinical Microbiology as part of standard clinical care. Culture results were recorded by the study investigators.

Definitions

PPE is defined as a patient meeting the inclusion criteria for CAP as above, with a new effusion on chest radiograph as recorded by the reporting radiologist. This definition concurs with the British Thoracic Society pleural guidelines,[24] and can represent a minimum of around 200mls of pleural fluid.[25] Pleural ultrasound was not performed on every patient unless requested and performed by the relevant clinical team. Complicated PPE is defined as any effusion requiring intercostal chest drainage (including surgical therapy if tube drainage was not deemed possible).

Patients were defined as having pneumococcal CAP if any microbiological test was positive for *S. pneumoniae*, including blood, pleural fluid, or sputum culture, Binax NOW®, or Bio-Plex serotype-specific antigen detection. Patients with pneumococcal CAP where no serotype was detected after testing with the Bio-Plex assay (i.e. *S. pneumoniae* detected by Binax NOW® or sputum culture, but no serotype determined by Bio-Plex or blood culture) were described as having "untyped" pneumococcal CAP. Serotypes included in PCV-7 (4, 6B, 9V, 14, 18C, 19F, 23F) are hereafter referred to as "PCV-7 vaccine-type (VT)", and serotypes not contained within PCV-7 as "non vaccine-type" (NVT). Serotypes included in PCV-13 (1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 23F) are hereafter referred to as "PCV-13 VT". Serotypes associated with PPE in paediatric cohorts (1, 3, 7F and 19A) are defined as "PPE-related serotypes". All PCV-7 and PCV-13 VT serotypes are detectable by the Bio-Plex assay. Highly invasive serotypes were defined as 1, 5, 7F and 8 according to previous publications,[22] with the remainder defined as less invasive serotypes.

Analysis

Statistical calculations were made using SPSS v16.0 (©SPSS Inc., 1989-2007). Categorical data were compared using Pearson's χ^2, which was also used for calculating odds ratios (OR) and 95% confidence intervals (CI). Continuous data that were non-normally distributed were compared using Mann-Whitney U test. Differences in 30-day mortality, pneumonia severity (as measured by the Pneumonia Severity Index (PSI))[3] and length of hospital stay (LOS) were investigated between patients with PPE and patients with CAP in the absence of PPE. Within the sub-group of patients with pneumococcal CAP, associations were investigated between CAP caused by PPE-related serotypes and the development of PPE, and which (if any) individual serotypes had a predisposition to causing PPE.

To adjust for potential confounding variables, we performed a multivariable logistic regression analysis. Although no previous publications have identified risk factors for

pneumococcal PPE, we inferred potential risk factors from studies identifying risk factors for complicated PPE and complicated CAP (defined as multi-lobar CAP or CAP with PPE or empyema).[13-15] These include age <60 years, tachycardia >100 beats per minute, leucocytosis >15,000 mm^{-3}, C-reactive protein (CRP) >100 mg/l, presence of chronic liver disease or COPD, serum creatinine >130 µmol/l, sodium <130 mmol/l, and platelet count >400×10^9/l. (Note that pleuritic pain, albumin <30 g/l and alcohol abuse, although identified as potential risk factors for complicated PPE, were not recorded in all patients and therefore not included in the analysis). Analyses were also performed to adjust for severity of CAP using PSI risk group.[3]

Results

Of 1100 patients identified with CAP during the study period, 956 consented to be included in the study. Thirty-six patients were unable to provide a urine sample (and had no other test positive for pneumococcus), leaving 920 for analysis. The baseline demographics and clinical characteristics of the study population are described in table 1. Of the 920 participants enrolled in the study, 366 were found to have pneumococcal aetiology. Of these, 100/366 (27.3%) were associated with PPE at presentation compared with 109/554 (19.7%) patients with non-pneumococcal aetiology (OR 1.5, 95% CI 1.1-2.1; p=0.007). This association was maintained after adjustment for disease severity (OR 1.5, 95% CI 1.1-2.1; p=0.010). Of 209 patients with all-cause PPE, 56 (26.8%) had complicated PPE; comprising 30 of 100 (30%) patients with pneumococcal PPE compared with 26 of 109 (23.9%) with non-pneumococcal PPE. (OR 1.8, 95% CI 1.1-3.1; p=0.029). Thoracocentesis was performed in 83 of 209 PPEs (39.7%); pleural fluid culture was positive for pneumococcus in 2 patients.

PPE and outcome

All-cause PPE was associated with higher 30-day mortality compared to patients without PPE (29/209 (13.9%) versus 63/711 (8.9%); OR 1.7, 95% CI 1.0-2.7; p=0.034) on univariate analysis but not after adjustment for disease severity (OR 1.4, 95% CI 0.9-2.3; p=0.15). The incidence of PPE increased with increasing pneumonia severity (PSI classes I-III: 68/395 (17.2%), PSI class IV: 89/336 (26.4%), PSI class V: 52/189 (27.5%); p value for trend 0.002). Median LOS in those who survived to discharge (n=832) was longer for patients with PPE (10 days, interquartile range (IQR) 5-17 days, versus 6 days, IQR 4-11 days; p<0.001).

Serotypes in adult pneumococcal PPE

A serotype was determined in 246 of 366 (67.2%) patients with pneumococcal CAP, including 40 patients with a positive blood culture. The prevalence of PPE was highest for patients with serotypes 19A (9/20; 45%), 1 (18/40; 45%) and 3 (8/20; 40%) (table 2). Of 94 adults with a serotype identified as likely to cause pneumococcal PPE from paediatric studies (serotypes 1, 3, 7F and 19A), 38 (40.4%) had PPE compared with 35 of 152 (23.0%) with other serotypes (unadjusted OR 2.3, 95% CI 1.3-4.0; p=0.004) and 27 of 120 (22.5%) with untyped pneumococcal CAP (OR 2.3, 95% CI 1.3-4.2; p=0.007) (figure 1, table 3). After adjustment for all putative risk factors, CAP due to PPE-related serotypes remained significantly associated with PPE when compared with other pneumococcal cases (OR 2.3, 95% CI 1.3-4.1; p=0.003), or the untyped group alone (OR 2.5, 95% CI 1.2 to 4.8, p=0.007 (table 4)).

Of the PPE-related serotypes, serotypes 1 and 19A were individually associated with PPE when compared with the untyped group (serotype 1: adjusted OR 2.8, 95% CI 1.3-6.0, p=0·007; serotype 19A: adjusted OR 2·8, 95% CI 1·1-7·5, p=0·038). These associations were maintained when adjusted for disease severity (serotype 1: adjusted OR 3.1, 95% CI 1·5-6.8, p=0·004; serotype 19A: adjusted OR 3.1, 95% CI 1·1-8.2; p=0·027) or adjusted for putative risk factors for pneumococcal PPE (serotype 1: adjusted OR 2.4, 95% CI 1.1-5.5, p=0.034; serotype 19A: adjusted OR 2.7, 95% CI 1.0-7.7; p=0·061). Only six serotypes occurred in cases of complicated pneumococcal PPE; 1 (n=9), 19A (n=4), 3, 4, 8 and 14 (all n=2).

Patients with a pneumococcal serotype not included within PCV-7 (n=170) were more likely to present with PPE compared to those with a PCV7 serotype (58/170 (34.1%) versus 14/72 (19.4%), OR 2.1, 95% CI 1.1-4.2; p=0.024). There was no evidence for a difference in the odds of PPE in patients with PCV-13 VT serotype compared to a combined group of those with a serotype not included in PCV-13 or with untyped pneumococcal CAP (n=155; OR 0.8, 95% CI 0.5-1.2; p=0.301). Highly invasive serotypes (1, 5, 7F and 8) were positively associated with PPE (38/107 (35.5%) versus 62/259 (23.9%), OR 1.8, 95% CI 1.1-2.8; p=0.024), but not complicated PPE (12/38 (31.5%) versus 18/62 (29.0%), OR 1.1, 95% CI 0.5-2.7; p=0.787).

Discussion

The main findings from this study are that PPE and complicated PPE are strongly associated with pneumococcal aetiology, and the serotypes independently associated with adult PPE are similar to those seen in childhood PPE, namely serotypes 1, 3, 7F and 19A. In particular, serotypes 1 and 19A are strongly associated with adult PPE. Pneumococcal serotype can have profound implications for the spectrum of clinical disease, and has been shown to influence 30-day mortality, invasive disease potential, and disease severity.[26-28] To our knowledge, this is the only study in adults with CAP to investigate the relationship between pneumococcal serotype and both invasive and non-invasive pneumococcal PPE.

In the only other related study in adults,[13] serotypes were only available in patients who had invasive pneumococcal disease (n=84). That study identified serotypes 1, 3 and 19A as being most frequently found in "complicated CAP" which included multi-lobar disease as well as PPE and empyema. Paediatric PPE increased in incidence following PCV-7 introduction and prior to the introduction of PCV-13,[11, 18-20] with PCV-7 NVT serotypes 1, 3, 5, 7F and 19A implicated.[10-12] In a small study of paediatric empyema, ten serotypes were identified, six of which were serotype 19A and one serotype 1, the others being 14 (n=1), 34 (n=1) and untypable (n=4).[29] In another study, serotype 1 was particularly associated with complicated CAP (defined as PPE, empyema or necrotizing CAP) in children when compared with other serotypes.[30] No data are yet available on the serotype distribution of PPE since PCV-13 was introduced in 2010.

The serotypes identified as associated with PPE and complicated PPE in this study are similar to the findings of studies conducted in infants and children.[11, 12] This raises the suggestion that for serotypes 1, 3, 7F and 19A, pathogen-related factors may be more important in the pathogenesis of PPE compared to age or host-related factors. The mechanisms whereby these serotypes might cause PPE are unclear. Zwitterionic polysaccharides contained within the serotype 1 coat can directly activate T helper cells in a similar way to proteins via a major histocompatibility complex (MHC) class II-dependent pathway.[31] Additionally, it has been suggested that serotypes with a lower degree of encapsulation have a higher degree of interaction with the respiratory epithelium, and hence cause invasive disease, of which PPE and empyema may be a manifestation.[32] Serotypes with a low degree of encapsulation include 1, 4, 5, 7F and 14, with 19A of intermediate thickness. However, serotype 3 is heavily encapsulated, suggesting that there may be other significant interacting mechanisms underlying aetiology of PPE. Serotype also affects the amount of complement deposition and neutrophil phagocytosis,[33] and adhesion to and

invasion through the respiratory epithelium via variable exposure of bacterial proteins,[34] although serotypes 1 and 19A have not been individually studied in this regard. More studies are required to fully explore this interaction between capsule type and host defence.

Of the previously described putative clinical risk factors for pneumococcal PPE, younger age was found to be independently associated with pneumococcal PPE. Interestingly, pleuritic chest pain is more commonly reported in younger patients with pneumococcal CAP than in older patients,[35] and has previously been associated with the development of PPE.[15, 15] The observed increased incidence of PPE and infection with PPE-related serotypes in younger patients provides a potential explanation for this finding, though the underlying mechanisms are unclear. Tachycardia was unexpectedly found to be negatively associated with PPE in the cohort with pneumococcal CAP, although it was not associated with all-cause PPE. In contrast Falquera and colleagues reported an independent association of tachycardia >100 beats per minute with all-cause empyema and complicated PPE.

The outcomes of patients with PPE have consistently been shown to be worse than those without PPE, and this study confirms these findings. In particular, length of stay is significantly longer for patients with PPE. Effusions requiring chest tube drainage would be expected to lengthen median length of stay, as would a higher proportion of more severe or more inflammatory disease. In addition, radiographic resolution of PPE may take longer than uncomplicated consolidation, thereby lengthening the duration of symptom recovery.

Following the introduction of conjugate vaccines to national childhood vaccination schedules, a fall in the incidence of invasive pneumococcal disease across all age groups together with a significant change in the serotype distribution has been observed.[27] In particular, serotypes 1 and 19A, along with other PCV-7 NVT serotypes increased in prevalence following the introduction of PCV-7 vaccination. The additional serotypes contained within PCV-13 (1, 3, 5, 6A, 7F and 19A) include those identified in this study as implicated in the development of pneumococcal PPE. Therefore a potential unintended benefit of the introduction of PCV-13 may be a fall in the incidence of pneumococcal PPE over the next few years. However, further serotype replacement consequent on PCV-13 vaccination may mean that previously less common serotypes may emerge with an as yet unknown clinical impact. This underlines the importance of ongoing serotype surveillance, particularly for complicated CAP.

Study limitations

A number of limitations to the study have been discussed in a previous publication.[22] The main limitation of the current study is that a pleural fluid sample was not taken for microbiological analyses from all cases with a PPE. Thoracocentesis was performed at the discretion of the attending clinical team, and where not performed this is likely to be due to small size of the PPE and where chest drainage was not felt to be clinically imperative. This also limits the ability to differentiate the PPEs into its different stages. The assumption is made that the effusions present on chest radiograph are related to the concurrent consolidation, and are of the same aetiology and serotype. It would seem unlikely that a new effusion in combination with consolidation on chest radiograph would be unrelated, but we cannot exclude this possibility. Secondly, the putative risk factors for pneumococcal PPE that were examined in this study were based on two unvalidated studies; one identified risk factors for complicated pneumococcal CAP and the other all-cause complicated PPE. Therefore there may be other potential confounders that might account for these findings.

Conclusion

Serotypes 1, 3, 7F and 19A are independently associated with adult PPE, a similar finding to childhood PPE. This suggests that pathogen factors are important in the development of PPE and that serotype replacement following pneumococcal vaccination strategies may impact on the spectrum of clinical disease.

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Competing interests

TB has received a salary derived from an unrestricted grant from Wyeth (now Pfizer). CS has received support for travel to meetings for other purposes from Pfizer. SG has received a salary derived from an unrestricted grant from Wyeth (now Pfizer). MS has received support for travel to meetings for other purposes and is on advisory boards for Wyeth (now Pfizer), Merck and GlaxoSmithKline, and has received grants from GlaxoSmithKline and Pfizer. MS has also spoken at scientific meetings organised by Pfizer and GlaxoSmithKline. VM declares no conflict of interest. CT has received a grant from the National Institute of Health Research (NIHR) as part of a personal post-doctoral fellowship. RG has received an unrestricted grant from Wyeth (now Pfizer), and has received support for travel to meetings for other purposes and grants from Wyeth (now Pfizer) and GlaxoSmithKline. WSL has received an unrestricted research grant from Pfizer.

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Tables

Patient characteristics	Whole cohort (n=920)	PPE (n=209)	No PPE (n=711)	P value*	Complicated PPE (n=56)
Demographics					
Age, median; years (IQR)	71.7 (57.8-80.8)	70.7 (51.0-79.9)	72.0 (59.1-80.5)	0.096	51.3 (38.7-68.9)
Male (%)	546 (59.3)	116 (55.5)	430 (60.5)	0.198	33 (58.9)
Residential or nursing care home resident (%)	51 (5.5)	8 (3.8)	43 (6.0)	0.222	1 (1.8)
WHO Performance Status ≥2 (%)	138 (15.0)	28 (13.4)	118 (16.6)	0.277	5 (8.9)
COPD (%)	244 (26.5)	49 (23.4)	195 (27.4)	0.252	7 (12.5)
Ischaemic heart disease (%)	145 (15.8)	28 (13.4)	118 (16.6)	0.625	7 (12.5)
Diabetes mellitus (%)	130 (14.1)	35 (16.7)	95 (13.4)	0.200	8 (14.2)
Cerebrovascular disease (%)	103 (11.2)	26 (12.4)	77 (10.8)	0.506	6 (10.7)
Asthma (%)	102 (11.1)	28 (13.4)	74 (10.4)	0.226	5 (8.9)
Congestive cardiac failure (%)	74 (8.0)	20 (9.6)	54 (7.6)	0.349	4 (7.1)
Active malignancy (%)	67 (7.3)	13 (6.2)	54 (13.4)	0.509	4 (7.1)
Dementia (%)	32 (3.5)	5 (2.4)	27 (3.8)	0.329	0 (0)
Mean Charlson co-morbidity index (95% CI)	1.48 (1.37-1.59)	1.57 (1.32-1.81)	1.45 (1.33-1.57)	0.734	1.17 (0.67-1.67)
Influenza vaccination in preceding 12 months (%)	558/855 (65.3)	118/194 (60.8)	440/661 (66.6)	0.140	25/54 (46.3)
PPV in preceding 10 years (%)	383/824 (46.5)	85/191 (44.5)	298/633 (47.1)	0.532	14/53 (26.4)
Severity					
PSI Class I-III (%)	395 (42.9)	68 (32.5)	327 (46.0)		31 (55.4)
PSI Class IV (%)	336 (36.5)	89 (42.6)	247 (34.7)	0.002	18 (32.1)
PSI Class V (%)	189 (20.5)	52 (24.9)	137 (19.3)		7 (12.5)
Aetiology					
Pneumococcal	366 (39.8)	100 (47.8)	266 (37.4)	0.007	30 (53.6)
Non-pneumococcal	554 (60.2)	109 (51.2)	445 (62.6)		26 (46.4)
Outcome					
30-day mortality (%)	92 (10.0)	29 (13.9)	63 (8.8)	0.034	4 (7.1)
LOS, median; days (IQR)	7 (4-12)	10 (5-17)	6 (4-11)	<0.001	13 (5-15)
IRVS (%)	82 (8.9)	23 (11.0)	59 (8.3)	0.227	5 (8.9)

PPE: para-pneumonic effusion. PPV: adult pneumococcal polysaccharide vaccine; LOS: length of hospital stay; IRVS: need for intensive respiratory or vasopressor support; COPD: chronic obstructive pulmonary disease; CI: confidence interval; WHO: World Health Organisation; PSI: pneumonia severity index; IQR: interquartile range. *P value compares patients with PPE with those without PPE.

Table 1: Characteristics and outcomes of the study cohort presenting with community-acquired pneumonia (all cause).

Serotype	All pneumococcal (n=366)	Pneumococcal PPE (%) (n=100)	Complicated pneumococcal PPE (%) (n=30)	Multi-lobar (%) (n=116)
1	40	18 (45)	9 (23)	9 (23)
3	20	8 (40)	2 (10)	7 (35)
4	13	3 (23)	2 (15)	4 (31)
5	18	6 (33)	0 (0)	6 (33)
6A/C	11	1 (9)	0 (0)	4 (36)
6B	2	0 (0)	0 (0)	1 (50)
7F	14	3 (21)	1 (7)	4 (29)
8	35	11 (31)	2 (6)	10 (29)
9V	4	1 (25)	0 (0)	1 (25)
14	45	8 (18)	2 (4)	20 (44)
18C	4	0 (0)	0 (0)	0 (0)
19A	20	9 (45)	4 (20)	7 (35)
19F	3	1 (33)	0 (0)	1 (33)
23F	0	0 (0)	0 (0)	0 (0)
Other	17	4 (24)	1 (6)	5 (29)
Untyped	120	27 (23)	7 (6)	37 (28)

PPE: para-pneumonic effusion.

Table 2. Proportion of cases caused by each serotype by disease site.

	No PPE	PPE			
	Pneumococcal CAP	**PPE-associated serotypes (1, 3 7F, 19A)**	**Other serotypes**	**OR (95% CI)***	**p value***
	n=266	**n=38**	**n=62**		
Clinical feature at presentation					
Age (years; median, IQR)	72.2 (57.9-82.1)	65.0 (42.0-78.7)	65.3 (47.6-78.7)	0.99 (0.98-1.02)	0.900
CCI (mean, 95% CI)	1.61 (1.39-1.83)	1.21 (0.73-1.69)	1.69 (1.22-2.17)	0.87 (0.75-1.01)	0.064
COPD (%)	70 (26.3)	10 (26.3)	14 (22.6)	1.22 (0.48-3.12)	0.671
Asthma (%)	32 (12.0)	3 (7.9)	12 (19.4)	0.36 (0.09-1.36)	0.119
Creatinine (µmol/l; median, IQR)	101 (75-143)	90 (76-131)	93 (70-159)	1.00 (0.99-1.00)	0.319
CRP (mg/l; median, IQR)	165 (79-262)	260 (93-345)	176 (79-278)	1.00 (1.00-1.01)	0.197
Complicated PPE (%)	0 (0)	16 (42.1)	14 (22.6)	2.49 (1.04-5.99)	**0.039**
Bacteraemia (%)	28 (10.5)	8 (21.1)	4 (6.5)	3.87 (1.08-13.89)	**0.029**
Shock (%)	32 (12.0)	6 (15.8)	6 (9.7)	1.12 (0.36-3.44)	0.845
PSI class					
I-III (%)	117 (44.0)	12 (31.6)	22 (35.5)		
IV (%)	92 (34.6)	17 (44.7)	23 (37.1)	-	0.750
V (%)	57 (21.4)	9 (23.7)	17 (27.4)		
Outcomes					
IRVS (%)	29 (10.9)	4 (10.5)	10 (16.1)	0.61 (0.18-2.11)	0.433
30-day mortality (%)	23 (8.6)	4 (10.5)	8 (12.9)	0.79 (0.22-2.84)	0.723

PPE: para-pneumonic effusion; CAP: community-acquired pneumonia; IQR: interquartile range; CCI: Charlson Co-morbidity Index; CI: confidence interval; CRP: C-reactive protein; COPD: chronic obstructive pulmonary disease; PSI: pneumonia severity index; IRVS: invasive respiratory or vasopressor support. PPE-associated serotypes include 1, 3, 7F and 19A. Shock is defined as systolic blood pressure less than 90 mmHg. *: Comparison between PPE-associated serotypes and Other serotypes

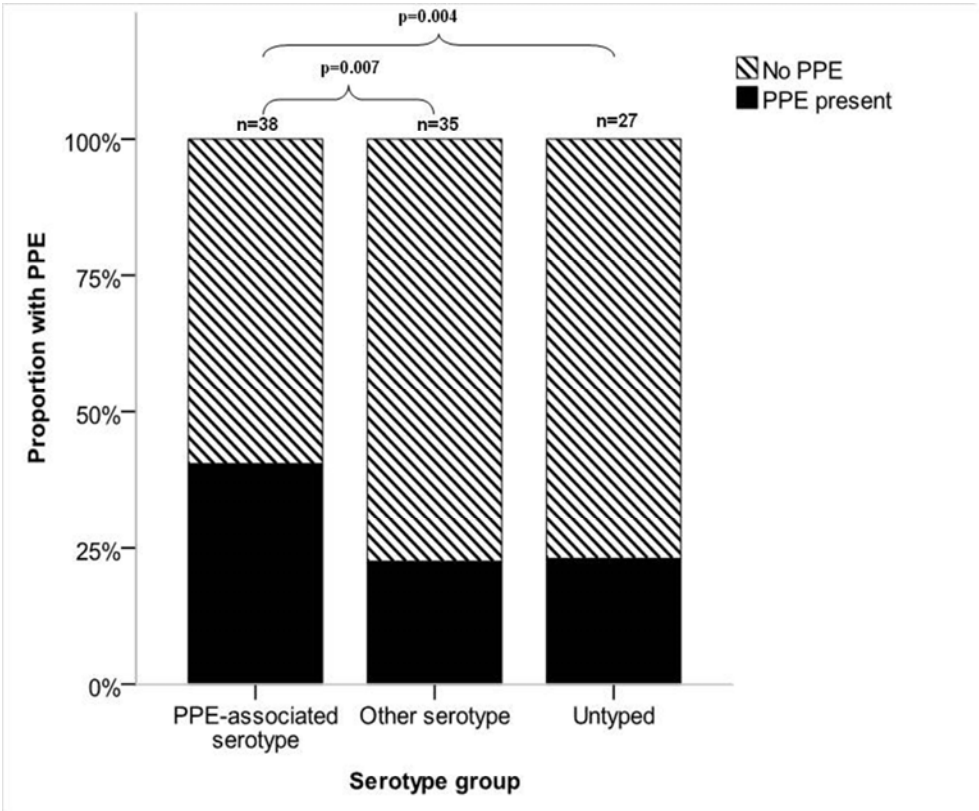
Table 3. Comparison of clinical characteristics and outcomes of patients with pneumococcal CAP.

	OR	95% CI	P value
Age <60 years	2.7	1.5-4.9	**0.001**
PPE-related serotype	2.5	1.3-4.8	**0.007**
Other serotype	1.1	0.6-2.0	0.766
Creatinine >130 µmol/l	1.1	0.6-2.0	0.674
CRP >100 mg/l	1.0	0.5-1.7	0.897
COPD	1.6	0.8-3.0	0.189
Chronic liver disease	0.7	0.1-3.7	0.695
Sodium <130 mmol/l	1.7	0.8-3.6	0.141
Platelets >400×10^9/l	1.9	0.7-4.6	0.207
Tachycardia >100 beats min^{-1}	0.5	0.3-0.9	**0.012**
Leucocytes >15,000 mm^{-3}	0.7	0.4-1.2	0.160

OR: odds ratio; CI: confidence interval; COPD: chronic obstructive pulmonary disease; CRP: C-reactive protein. "PPE-related serotypes" comprise 1, 3, 7F and 19A. "Other serotypes" comprise the group of all other detectable serotypes, excluding the untyped group.

Table 4. Multivariate logistic regression analysis of hypothesised risk factors for pneumococcal PPE, with untyped pneumococcal CAP as a reference group.

Figure 1



PPE: para-pneumonic effusion. "PPE-associated serotypes" include 1, 3, 7F, and 19A.

Figure 1. Proportion of each pneumococcal serotype group with PPE.

References

[1] Sahn SA. Diagnosis and management of parapneumonic effusions and empyema. Clin Infect Dis. 2007 Dec;45(11):1480–1486. Available from: http://dx.doi.org/10.1086/-522996.

[2] Lim WS, van der Eerden MM, Laing R, Boersma WG, Karalus N, Town GI, et al. Defining community acquired pneumonia severity on presentation to hospital: an international derivation and validation study. Thorax. 2003 May;58(5):377–382.

[3] 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 Jan;336(4):243–250.

[4] Hasley PB, Albaum MN, Li YH, Fuhrman CR, Britton CA, Marrie TJ, et al. Do pulmonary radiographic findings at presentation predict mortality in patients with community-acquired pneumonia? Arch Intern Med. 1996 Oct;156(19):2206–2212.

[5] Fine NL, Smith LR, Sheedy PF. Frequency of pleural effusions in *Mycoplasma* and viral pneumonias. N Engl J Med. 1970 Oct;283(15):790–793. Available from: http://-dx.doi.org/10.1056/NEJM197010082831505.

[6] Taryle DA, Potts DE, Sahn SA. The incidence and clinical correlates of parapneumonic effusions in pneumococcal pneumonia. Chest. 1978 Aug;74(2):170–173.

[7] Cillóniz C, Ewig S, Polverino E, Marcos MA, Esquinas C, Gabarrús A, et al. Microbial aetiology of community-acquired pneumonia and its relation to severity. Thorax. 2011 Apr;66(4):340–346. Available from: http://dx.doi.org/10.1136/thx.2010.143982.

[8] Lim WS, Macfarlane JT, Boswell TC, Harrison TG, Rose D, Leinonen M, et al. Study of community acquired pneumonia aetiology (SCAPA) in adults admitted to hospital: implications for management guidelines. Thorax. 2001 Apr;56(4):296–301.

[9] Park IH, Pritchard DG, Cartee R, Brandao A, Brandileone MCC, Nahm MH. Discovery of a new capsular serotype (6C) within serogroup 6 of *Streptococcus pneumoniae*. J Clin Microbiol. 2007 Apr;45(4):1225–1233. Available from: http://dx.doi.org/-10.1128/JCM.02199-06.

[10] Spencer D, Thomas M, Mohammed E, Clark J, Rushton S, Paton J. National surveillance of paediatric empyema in the UK; the UK-ESPE study. Presented as an abstract at the European Respiratory Society Annual Congress, Vienna. 2012;P2946.

[11] Byington CL, Hulten KG, Ampofo K, Sheng X, Pavia AT, Blaschke AJ, et al. Molecular epidemiology of pediatric pneumococcal empyema from 2001 to 2007 in Utah. J

Clin Microbiol. 2010 Feb;48(2):520–525. Available from: http://dx.doi.org/10.1128/-JCM.01200-09.

[12] Yu J, Salamon D, Marcon M, Nahm MH. Pneumococcal serotypes causing pneumonia with pleural effusion in pediatric patients. J Clin Microbiol. 2011 Feb;49(2):534–538. Available from: http://dx.doi.org/10.1128/JCM.01827-10.

[13] Cillóniz C, Ewig S, Polverino E, Muñoz-Almagro C, Marco F, Gabarrús A, et al. Pulmonary complications of pneumococcal community-acquired pneumonia: incidence, predictors, and outcomes. Clin Microbiol Infect. 2011 Oct;Available from: http://dx.doi.org/-10.1111/j.1469-0691.2011.03692.x.

[14] Chalmers JD, Singanayagam A, Murray MP, Scally C, Fawzi A, Hill AT. Risk factors for complicated parapneumonic effusion and empyema on presentation to hospital with community-acquired pneumonia. Thorax. 2009 Jul;64(7):592–597. Available from: http://-dx.doi.org/10.1136/thx.2008.105080.

[15] Falguera M, Carratalà J, Bielsa S, García-Vidal C, Ruiz-González A, Chica I, et al. Predictive factors, microbiology and outcome of patients with parapneumonic effusion. Eur Respir J. 2011 Nov;38(5):1173–1179. Available from: http://dx.doi.org/10.1183/-09031936.00000211.

[16] Miller E, Andrews NJ, Waight PA, Slack MP, George RC. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate vaccination in England and Wales: an observational cohort study. Lancet Infect Dis. 2011 Oct;11(10):760–768. Available from: http://dx.doi.org/10.1016/S1473-3099(11)70090-1.

[17] Lexau CA, Lynfield R, Danila R, Pilishvili T, Facklam R, Farley MM, et al. Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine. JAMA. 2005 Oct;294(16):2043–2051.

[18] Hendrickson DJ, Blumberg DA, Joad JP, Jhawar S, McDonald RJ. Five-fold increase in pediatric parapneumonic empyema since introduction of pneumococcal conjugate vaccine. Pediatr Infect Dis J. 2008 Nov;27(11):1030–1032. Available from: http://dx.doi.org/-10.1097/INF.0b013e31817e5188.

[19] Grijalva CG, Zhu Y, Nuorti JP, Griffin MR. Emergence of parapneumonic empyema in the USA. Thorax. 2011 Aug;66(8):663–668. Available from: http://dx.doi.org/10.1136/-thx.2010.156406.

[20] Li STT, Tancredi DJ. Empyema hospitalizations increased in US children despite pneumococcal conjugate vaccine. Pediatrics. 2010 Jan;125(1):26–33. Available from: http://-dx.doi.org/10.1542/peds.2009-0184.

[21] Koshy E, Murray J, Bottle A, Sharland M, Saxena S. Impact of the seven-valent pneumococcal conjugate vaccination (PCV7) programme on childhood hospital admissions

for bacterial pneumonia and empyema in England: national time-trends study, 1997-2008. Thorax. 2010 Sep;65(9):770–774. Available from: http://dx.doi.org/10.1136/thx.2010.137802.

[22] Bewick T, Sheppard C, Greenwood S, Slack M, Trotter C, George R, et al. Serotype prevalence in adults hospitalised with pneumococcal non-invasive community-acquired pneumonia. Thorax. 2012 Jun;67(6):540–545. Available from: http://dx.doi.org/10.1136/-thoraxjnl-2011-201092.

[23] Sheppard CL, Harrison TG, Smith MD, George RC. Development of a sensitive, multiplexed immunoassay using xMAP beads for detection of serotype-specific *Streptococcus pneumoniae* antigen in urine samples. J Med Microbiol. 2011 Jan;60(Pt 1):49–55. Available from: http://dx.doi.org/10.1099/jmm.0.023150-0.

[24] Maskell NA, Butland RJA, Pleural Diseases Group SoCCTS. BTS guidelines for the investigation of a unilateral pleural effusion in adults. Thorax. 2003 May;58 Suppl 2:ii8–i17.

[25] Blackmore CC, Black WC, Dallas RV, Crow HC. Pleural fluid volume estimation: a chest radiograph prediction rule. Acad Radiol. 1996 Feb;3(2):103–109.

[26] Luján M, Gallego M, Belmonte Y, Fontanals D, Vallès J, Lisboa T, et al. Influence of pneumococcal serotype group on outcome in adults with bacteraemic pneumonia. Eur Respir J. 2010 Nov;36(5):1073–1079. Available from: http://dx.doi.org/10.1183/-09031936.00176309.

[27] Miller E, Andrews NJ, Waight PA, Slack MP, George RC. Herd immunity and serotype replacement 4 years after seven-valent pneumococcal conjugate vaccination in England and Wales: an observational cohort study. Lancet Infect Dis. 2011 May;Available from: http://dx.doi.org/10.1016/S1473-3099(11)70090-1.

[28] van Hoek AJ, Andrews N, Waight PA, George R, Miller E. Effect of serotype on focus and mortality of invasive pneumococcal disease: coverage of different vaccines and insight into non-vaccine serotypes. PLoS One. 2012;7(7):e39150. Available from: http://dx.doi.org/-10.1371/journal.pone.0039150.

[29] Lee JH, Kim SH, Lee J, Choi EH, Lee HJ. Diagnosis of pneumococcal empyema using immunochromatographic test on pleural fluid and serotype distribution in Korean children. Diagn Microbiol Infect Dis. 2012 Feb;72(2):119–124. Available from: http://-dx.doi.org/10.1016/j.diagmicrobio.2011.09.025.

[30] Resti M, Moriondo M, Cortimiglia M, Indolfi G, Canessa C, Becciolini L, et al. Community-acquired bacteremic pneumococcal pneumonia in children: diagnosis and serotyping by real-time polymerase chain reaction using blood samples. Clin Infect Dis. 2010 Nov;51(9):1042–1049. Available from: http://dx.doi.org/10.1086/656579.

[31] Velez CD, Lewis CJ, Kasper DL, Cobb BA. Type I *Streptococcus pneumoniae* carbohydrate utilizes a nitric oxide and MHC II-dependent pathway for antigen presentation.

Immunology. 2009 May;127(1):73–82. Available from: http://dx.doi.org/10.1111/j.1365-2567.2008.02924.x.

[32] Weinberger DM, Trzci?ski K, Lu YJ, Bogaert D, Brandes A, Galagan J, et al. Pneumococcal capsular polysaccharide structure predicts serotype prevalence. PLoS Pathog. 2009 Jun;5(6):e1000476. Available from: http://dx.doi.org/10.1371/-journal.ppat.1000476.

[33] Hyams C, Camberlein E, Cohen JM, Bax K, Brown JS. The *Streptococcus pneumoniae* capsule inhibits complement activity and neutrophil phagocytosis by multiple mechanisms. Infect Immun. 2010 Feb;78(2):704–715. Available from: http://dx.doi.org/-10.1128/IAI.00881-09.

[34] Sanchez CJ, Hinojosa CA, Shivshankar P, Hyams C, Camberlein E, Brown JS, et al. Changes in capsular serotype alter the surface exposure of pneumococcal adhesins and impact virulence. PLoS One. 2011;6(10):e26587. Available from: http://dx.doi.org/10.1371/-journal.pone.0026587.

[35] Esposito AL. Community-acquired bacteremic pneumococcal pneumonia. Effect of age on manifestations and outcome. Arch Intern Med. 1984 May;144(5):945–948.