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Serotype-specific mortality from invasive *Streptococcus pneumoniae* disease revisited

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Abstract

Background: Invasive infection with *Streptococcus pneumoniae* (pneumococci) causes significant morbidity and mortality. Case series and experimental data have shown that the capsular serotype is involved in the pathogenesis and a determinant of disease outcome.

Methods: Retrospective review of 464 cases of invasive disease among adults diagnosed between 1990 and 2001. Multivariate Cox proportional hazard analysis.

Results: After adjustment for other markers of disease severity, we found that infection with serotype 3 was associated with an increased relative risk (RR) of death of 2.54 (95% confidence interval (CI): 1.22–5.27), whereas infection with serotype 1 was associated with a decreased risk of death (RR 0.23 (95% CI, 0.06–0.97)). Additionally, older age, relative leucopenia and relative hypothermia were independent predictors of mortality.

Conclusion: Our study shows that capsular serotypes independently influenced the outcome from invasive pneumococcal disease. The limitations of the current polysaccharide pneumococcal vaccine warrant the development of alternative vaccines. We suggest that the virulence of pneumococcal serotypes should be considered in the design of novel vaccines.

Background

Streptococcus pneumoniae (pneumococci) is a leading cause of pneumonia, sepsis, and meningitis among adults. Mortality associated with invasive disease remains high at 5–35% depending on site of infection, age and comorbidity [1-4].

Ninety different capsular serotypes cause disease among humans but less than 30 types account for the majority (>90%) of invasive cases [5]. The capsular polysaccharide plays an important role in pneumococcal pathogenesis, e.g. its diversity allowed for immune evasion by preventing phagocytosis in non-immune individuals [6], the amount of capsular polysaccharide correlated with pneumococcal virulence [7,8], during experimental pneumococcal meningitis serotypes 3, 6B, 14, 23F caused more severe meningeal inflammation than serotypes 1, 5, 9 and 7F [9,10]. and in a murine model of pneumococcal sepsis

certain serotypes were more lethal than others [11]. In adult case series, unadjusted mortality rates from invasive disease were increased with serotypes 3 and 5 while infection with serotypes 1, 4, 9V 12F and 14 were associated with lower mortality rates [12-16].

Currently, a polysaccharide pneumococcal vaccine is recommended for elderly adults because it has been demonstrated to prevent invasive pneumococcal disease [17-20] However, the vaccine is unable to prevent non-bacteremic pneumonia. Future vaccine developments for adults are likely to be based on conjugate-polysaccharides and we believe that differences in serotype-specific mortality could be important to consider when including capsular serotypes.

The aim of the current study was to study serotype-specific mortality after adjustment for other factors associated with outcome.

Methods

Subjects

464 adult patients older than 16 years with first episodes of bacteremic pneumococcal disease diagnosed between January 1990 and January 2001 at Hvidovre University Hospital, Copenhagen were included. Cases were identified from the records of the Department of Clinical Microbiology.

Microbiology

Identification of *S. pneumoniae* and susceptibility testing for penicillin was done by the Department of Clinical Microbiology. All isolates were serotyped by the Quellung reaction at the Streptococcal Laboratory, Statens Serum Institute (SSI), Copenhagen, by using type-specific pneumococcal rabbit antisera [5]. Antimicrobial susceptibility testing was performed first as a screening with the agar disk diffusion test using oxacillin (1-μg disk; AB Biodisk, Solna, Sweden) and erythromycin (78 μg, Neo-Sensitabs; Rosco, Copenhagen, Denmark) on 10% horse blood agar plates (SSI). Isolates with reduced sensitivity against oxacillin or erythromycin were further characterized by determining the minimal inhibitory concentration (MIC) of penicillin, erythromycin, ceftriaxone, and ciprofloxacin by using the E-test (AB Biodisk) on resistance plates (4.6 mm; SSI).

Covariates

Data were extracted from patient records using a standardized data collection form. Information was collected at the time of diagnosis and included age, sex, site of infection, comorbidity, smoking status, alcoholism, splenectomy, residence, injection drug use, blood chemistry and in-hospital mortality. Comorbidity consisted of any of the following: chronic lung disease, chronic heart disease, chronic renal disease, chronic liver disease, diabetes mellitus, cancer, autoimmune disease, human immunodeficiency virus (HIV) infection, and 'other chronic diseases'. Alcoholism was defined as known abuse. Smoking was considered in two categories: never or ever. Results of chest roentgenograms were obtained and the presence of pulmonary infiltrates or pleural effusions noted.

Statistics

All values are expressed as median and interquartile range. Differences between groups were estimated by χ^2 statistics (Fisher's exact or Pearson's test, as appropriate). Survival curves were constructed by the method of Kaplan-Meier and compared by the log rank test. Relative risk (RR) with the 95% confidence interval (CI) for progression to death was estimated using univariate and multivariate Cox proportional hazard regression models. All variables with > 80% of values available were tested in univariate analysis and all variables with a P value less than 0.1 were entered in the multivariate analysis. Subjects with more than one episode of pneumococcal bacteremia were right censored, i.e. only their first episode was included in the analysis.

Statistical analyses were done using the Statistical Package for Social Sciences (version 11.0; SPSS; Chicago, IL).

Results

Patient characteristics

464 individual cases of invasive pneumococcal disease were identified. Baseline characteristics are given in table 1.

There were 123 deaths during hospitalization resulting in an in-hospital mortality rate of 26.5% (95% CI: 21.8–31.2%). 110 of 410 (26.8%) patients with a typed isolate died compared to 13 of 54 (24.1%) of patients without a typed isolate (P = 0.724). 51 (11.0%) patients required mechanical ventilation. Only five patients had a record of pneumococcal vaccination. In univariate analysis, survivors were younger, had less comorbidity, fewer had reported alcoholism, and had higher total blood leukocyte counts and temperature on admission (table 1). After multivariate analysis, increasing age, low temperature and a leukocyte count less than 9×10^9/L remained significantly associated with in-hospital mortality (table 3).

Capsular serotype and survival

37 different types were identified among 410 typed isolates (table 2). Two isolates could not be typed and were considered 'rough'. Mortality varied between serotype groups with type 3 having the highest unadjusted in-hospital mortality (50%). In contrast, the unadjusted mortality rates were between 0 and 10 % for serotypes 1, 6A and 20. Each serotype was entered separately in a Cox regression analysis and compared to all other cases. Only sero-

Table 1: Patient characteristics

	Mortality rate, %	Survivors No. (%)	Non-survivors No. (%)	P value
Age, years				
18–49	17.8	83 (25.9)	18 (14.6)	
50–65	33.8	47 (14.5)	24 (19.6)	
65–79	29.6	114 (35.5)	48 (39.0)	
> 80	30.0	77 (24.0)	33 (26.8)	0.077
Sex				
Female	29.4	199 (58.4)	64 (52.0)	
Male	24.3	142 (41.6)	59 (48.0)	0.244
Focus				
Bacteremia	35.4	62 (20.5)	34 (27.6)	
Pneumonia	27.1	226 (74.8)	84 (68.3)	
Meningitis	44.4	5 (1.7)	4 (0.8)	
Otitis media	10.0	9 (3.0)	1 (3.3)	0.153
Comorbidity*				
No	23.6	133 (43.8)	41 (33.3)	
Yes	32.4	171 (56.2)	82 (66.7)	0.047
Alcoholism				
No	27.0	265 (87.2)	98 (79.7)	
Yes	39.1	39 (12.8)	25 (20.3)	0.049
Smoking status				
Never	27.1	70 (29.8)	26 (33.3)	
Current or former	24.0	165 (70.2)	52 (66.7)	0.573
Splenectomy				
No	28.6	300 (98.7)	120 (97.6)	
Yes	42.9	4 (1.3)	3 (2.4)	0.416
Intravenous drug use				
No	29.4	293 (96.4)	122 (99.2)	
Yes	8.3	11 (3.6)	1 (0.8)	0.193
Nursing home residence				
Yes	27.6	21 (7.7)	8 (7.1)	
No	27.2	279 (92.3)	104 (92.9)	0.984
Temperature				
≥ 38.5°C	22.8	210 (71.9)	62 (56.4)	
< 38.5°C	36.9	82 (28.1)	48 (43.6)	0.003
B-leukocytes				
> 9 10^9/L	24.7	216 (81.8)	71 (65.7)	
≤ 9 10^9/L	43.5	48 (18.2)	37 (34.3)	0.001

*: includes patients with chronic lung disease, chronic heart disease, chronic renal disease, chronic liver disease, diabetes mellitus, cancer, autoimmune disease, human immunodeficiency virus (HIV) infection, and 'other chronic diseases'. Patients may have more than one comorbidity.

types 1 and 3 were significantly associated with in-hospital mortality. For the multivariable analysis, all types other than serotype 1 and 3 served as the reference group. Serotype 1 and 3 remained independently associated with mortality after adjusting for age, comorbidity, alcoholism, temperature and leukocyte count (table 3 and figure 1).

Analysis of outcome by primary focus

Pneumococcal bacteremic pneumonia

310 patients had a pulmonary focus. Of these, 84 (27.1%) died during hospitalization. 33 (10.6%) required mechanical ventilation. In univariate analysis, presence of a bilateral infiltrate was associated with an increased mortality (2.54 (1.60–4.03)) compared to a unilateral infiltrate, whereas the presence of an effusion was not. 235 patients with complete data were available for multivariate analysis. In this analysis, a bilateral infiltrate, age, alcoholism and leukocyte count were independently associated with mortality. Serotype 1 and 3 showed a trend towards an association with outcome at a significance level of 0.06 for both.

Pneumococcal bacteremia with unknown focus

96 patients had *S. pneumoniae* isolated from blood without a known focus. 34 (35.4%) patients died. Nine cases were caused by serotype 3 and of these six died. Five cases were caused by serotype 1. None of these were fatal. In univariate analysis, serotype 3 remained associated with

Table 2: *Streptococcus pneumoniae* capsular serotype associated mortality.

Serotype	No. (% of total)	Mortality (%)
1	46 (10.1)	4 (8.7)
3	24 (5.3)	12 (50)
4	49 (10.8)	11 (22.4)
6A	10 (2.2)	1 (10)
6B	12 (2.6)	5 (41.7)
7F	22 (4.8)	3 (13.6)
8	22 (4.8)	5 (22.7)
9N	13 (2.9)	4 (28.6)
9V	22 (4.8)	9 (39.1)
12F	18 (4.0)	5 (27.8)
14	37 (8.1)	9 (24.3)
19A	10 (2.2)	5 (50)
19F	12 (2.6)	4 (30.8)
20	14 (3.1)	
23F	15 (3.3)	5 (31.2)
5, 10A, 10F, 11A, 12A, 15A, 15B, 16F, 17F, 18C, 22F, 23A, 23B, 24F, 29, 33F, 34, 35F, 36, 38, 42, 48 and 'rough'	74 (15.9)	27 (36.5)
Not typed	54 (11.2)	13 (24.1)
Total	464 (100)	123 (26.5)

Table 3: Multivariate analysis of risk factors associated with in-hospital death from invasive pneumococcal infection

	Univariate RR (95% CI)	Multivariate RR (95% CI)*
Serotype		
Other	1.0	1.0
Type 3	2.14 (1.17–3.91)	2.54 (1.22–5.27)
Type 1	0.29 (0.11–0.77)	0.23 (0.06–0.97)
Age, years		
18–49	1.0	1.0
50–65	2.00 (1.08–3.68)	2.23 (1.00–4.97)
65–79	1.74 (1.01–2.99)	3.20 (1.50–6.81)
> 80	1.79 (1.01–3.18)	2.86 (1.23–6.65)
Comorbidity**		
No	1.0	1.0
Yes	1.44 (0.99–2.10)	1.24 (0.78–2.00)
Alcoholism		
No	1.0	1.0
Yes	1.55 (1.00–2.40)	1.82 (0.97–3.42)
B-leukocyte count		
> 9 10^9/L	1.0	1.0
≤ 9 10^9/L	2.07 (1.39–3.08)	2.76 (1.66–4.59)
Temperature		
≥ 38.5°C	1.0	1.0
< 38.5°C	1.79 (1.23–2.61)	1.67 (1.07–2.62)

*: Cox regression with all variables forced into the model. RRs are adjusted with all variables included in the model. **: includes patients with chronic lung disease, chronic heart disease, chronic renal disease, chronic liver disease, diabetes mellitus, cancer, autoimmune disease, human immunodeficiency virus (HIV) infection, and 'other chronic diseases'. Patients may have more than one comorbidity.

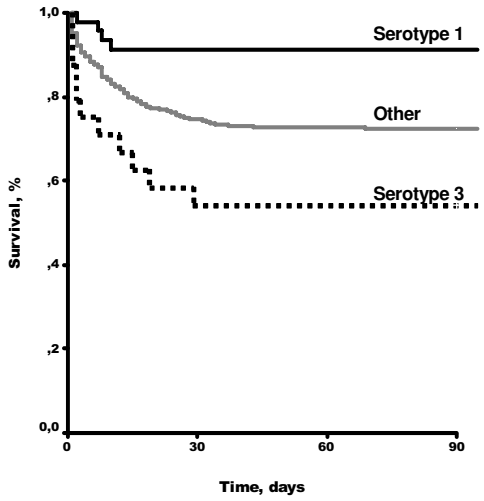

Figure 1
Cumulative survival according to serotype (log rank test: P = 0.006).

outcome (RR: 2.49, 95% CI: 1.02–6.10). In this subanalysis, leukocyte count was the only other variable associated with outcome by univariate analysis. Both remained significantly associated with outcome in multivariate analysis: serotype 3 (3.00 (1.12–8.08)) and leukocyte count less than 9 (4.15 (1.70–10.16)).

Pneumococcal meningitis and otitis media

Among nine cases of meningitis, none were caused by serotypes 1 and 3. Serotypes 4, 6B, 8, 10A, 12F, 14, and 19F accounted for one case each and serotype 24F for two cases.

Only ten cases of bacteremic otitis media were detected and of these, one case was caused by serotype 3. The patient survived. None were caused by serotype 1. Serotypes 4, 6A, 11A, 19A, 23F accounted for one case each and serotype 7F and 20 caused two cases each.

Antibiotic susceptibility

Five isolates (0.9%) had reduced susceptibility to penicillin. Only one isolate (serotype 14) had a MIC of penicillin ≥ 2 μg/ml. This patient survived but one patient with intermediate resistance died. Four isolates (serotypes 5, 9V, 14 and 19F) had an MIC of > 0.064 and < 2 μg/ml. Reduced susceptibility to erythromycin (MIC ≥ 4 μg/ml) was detected in 10 isolates (2.2%). Three of these patients died.

Discussion

In this study we show that pneumococcal capsular serotypes 1 and 3 predicted outcome from invasive disease regardless of age and other markers of disease severity. Additionally, we show that age and an inability to mount a rise in temperature or peripheral leukocytes independently predicted mortality.

Varying and complex repeating units of polysaccharide permit a large diversity among pneumococci. It has been known for decades that the capsule *per se* and the amount of capsule were necessary components of pneumococcal virulence [7,8]. Early work showed that the increased content of capsular polysaccharide of some pneumococci (e.g. serotype 3) increased resistance to phagocytosis [7,8]. Several groups have demonstrated that serotypes with high polysaccharide content generally were more lethal in murine models of sepsis than serotypes with lower polysaccharide content [11,12,22]. In addition, cell wall components and toxins (e.g. pneumolysin, autolysin, peptidoglycan and lipoteichoic acid) are thought to play a role in the inflammatory response during pneumococcal infection [23-25]. Whether the genetic background, the capsular type or both confer virulence is a matter of debate. Certainly the genetic background of the recipient strain plays a role since capsule transformed strains do not necessarily acquire the virulence of the donor strain [26]. Further, pneumococci switch capsules so strains with the same genetic background have heterologous capsular types and vice versa [27-29]. Henriques et al. studied clonality among serotype 3 and found that 24 of 29 strains were comprised of just two molecular patterns suggesting a close clonal relationship whereas other capsular types typically had several molecular patterns [16]. Unfortunately, molecular patterns were not studied in the present study. Future studies should investigate the prognostic value of molecular patterns as compared to capsular types.

As in numerous studies, increasing age [1,12,15,30,31], low temperature [32] and leucopenia [12,15,32,33] were factors associated with death from invasive disease although only few of these factors have been studied by multivariate analysis. Among patients with bacteremic pneumococcal pneumonia, we confirm that bilateral infiltrates increased the risk of death [32,34]. We did not find any association between smoking, nursing home residence, intravenous drug use, alcoholism, or comorbidity and risk of death. Mortality rates were comparable to studies published within the past 40 years [1,12-15,32,33,35]. Few patients had resistant strains and decreased suscepti-

bility to penicillin or erythromycin did not appear to influence outcome in this study.

Observational and case-control studies have shown that polysaccharide vaccination reduced the risk of invasive pneumococcal disease by approximately 60% among the elderly [17-20]. Less than 1% of our cohort had a record of pneumococcal vaccination in spite of a serotype coverage rate greater than 90%. However, the vaccine was introduced for general use for elderly people late (1996) in Denmark. Lack of efficacy against non-bacteremic pneumococcal pneumonia and the need for regular re-immunization remain a matter of concern. Therefore, alternative vaccine formulations for adults need to be evaluated. Recently, the introduction of a 7-valent conjugate vaccine has lead to a decrease in pneumococcal disease among children in the United States [36]. A 9-valent conjugate vaccine was shown to protect children against pneumonia and invasive disease [37]. Since the epidemiology of pneumococcal disease is different among adults, a conjugate vaccine would have to be composed differently in order to achieve acceptable coverage. In general, the frequency of serotypes recovered from invasive disease have been used to guide the development of pneumococcal vaccines although such an approach does not necessarily reflect the potential of a serotype to cause life-threatening disease. Conjugate vaccines are limited in the range of covered serotypes because of space constraints. In the Danish population the eleven most frequent serotypes (1, 3, 4, 6B, 7F, 8, 9N, 9V, 12F, 14 and 23F) account for 74% of invasive infections among adults and the thirteen most frequent (5 and 19F) account for 80% [5]. In this study, the current 7-valent conjugate would have a potential coverage rate of only 38%. In light of the current study, the life-threatening potential of a serotype should be taken into account when selecting serotypes for an adult conjugate vaccine. Larger studies are needed in order to rank serotypes accordingly.

This study has limitations because of its retrospective design. Several variables were unavailable from a number of cases leading to decreased statistical power of the multivariate analysis and possible introduction of bias. However, it is unlikely that there are any systematic differences between cases included in the final model and cases that were excluded.

Conclusion

Our study shows that capsular serotypes influence the outcome from invasive pneumococcal disease. The limitations of the current polysaccharide vaccine warrant the development of alternative vaccines such as a conjugate vaccine that may protect against non-bacteremic pneumonia [37]. We suggest that the virulence of pneumococcal serotypes should be considered in the design of novel vaccines.

Competing interests

None declared.

Authors' contributions

PM carried out the data collection, participated in the design of the study, the statistical analysis and drafted the manuscript. SWW participated in data collection and the design of the study. BL supervised antibiotic susceptibility testing. HBK supervised capsular serotyping. TB conceived the study, and participated in its design, analysis and coordination. All authors read and approved the final manuscript.

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