Prognostic factors of severe community-acquired staphylococcal pneumonia in France

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Shareable abstract (@ERSpublications)
In severe community-acquired staphylococcal pneumonia, Panton–Valentine leukocidin is associated with pleuropneumonia in toddlers and, in older patients, with increased mortality independent of methicillin resistance and other virulence factors https://bit.ly/3dQ5Dgm

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Abstract

Purpose *Staphylococcus aureus* causes severe forms of community-acquired pneumonia (CAP), namely staphylococcal pleuropneumonia in young children and staphylococcal necrotising pneumonia in older patients. Methicillin resistance and the Panton–Valentine leukocidin (PVL) toxin, as well as less specific factors, have been associated with poor outcome in severe CAP, but their roles are unclear.

Methods A prospective multicentre cohort study of severe staphylococcal CAP was conducted in 77 paediatric and adult intensive care units in France between January 2011 and December 2016. After age-clustering, risk factors for mortality, including pre-existing conditions, clinical presentation, laboratory features, strain genetic lineage, PVL, other virulence factors and methicillin resistance were assessed using univariate and multivariable Cox and LASSO (least absolute shrinkage and selection operator) regressions.

Results Out of 163 included patients, aged 1 month to 87 years, 85 (52.1%) had PVL-positive CAP; there were 20 (12.3%) patients aged <3 years (hereafter "toddlers"), among whom 19 (95%) had PVL-positive CAP. The features of PVL-positive CAP in toddlers matched with the historical description of staphylococcal pleuropneumonia, with a lower mortality (three (15%) out of 19) compared to PVL-positive CAP in older patients (31 (47%) out of 66). Mortality in older patients was predicted by PVL-positivity (hazard ratio (HR) 1.81, 95% CI 1.03–3.17) and methicillin resistance (HR 2.37, 95% CI 1.29–4.34) independently from *S. aureus* lineages and the presence of other determinants of virulence.

Conclusion PVL was associated with staphylococcal pleuropneumonia in toddlers and was a risk factor for mortality in older patients with severe CAP, independently of methicillin resistance, *S. aureus* genetic background and other virulence factors.

Introduction

Staphylococcal necrotising pneumonia caused by *Staphylococcus aureus* was first described in 1919 in adults during the influenza pandemic [1]. It is associated with airway haemorrhage, epithelial necrosis and a high fatality rate in otherwise healthy patients [2]. It was seldom reported during the 20th century [3, 4], until the epidemiological and pathophysiological links with Panton–Valentine leukocidin (PVL) were discovered at the turn of the century [2, 5]. Subsequent reports confirmed the high fatality rates (40–50%) of PVL-associated pneumonia in adults [6–9].

Independent of staphylococcal necrotising pneumonia, staphylococcal pleuropneumonia in young children was described in the late 1950s and 1960s as a specific clinical entity characterised by round-shaped lung infiltrations evolving towards bullous lesions, purulent pleural effusion and a lack of airway haemorrhage or epithelial necrosis [10, 11]. Recent case series of pleuropneumonia involving PVL-positive community-acquired *S. aureus* [12, 13] pointed to a possible relationship between pleuropneumonia and PVL; mortality was <5% in these series.

The striking differences between pleuropneumonia and necrotising pneumonia, both associated with PVL, but exhibiting contrasting mortality rates, may have contributed to controversies regarding the impact of PVL on the severity of staphylococcal pneumonia [14–16]. In this context, whether age affects both the clinical picture and the outcome of community-acquired PVL-positive staphylococcal pneumonia, which factors are associated with severity and whether PVL is an independent factor of severity are questions that remain to be answered and which are addressed herein.

Patients and methods

Ethics

The regional ethics committee approved the study (number: A11-39). Written informed consent was obtained from all patients or their parents.

Study design and participants

This is a prospective, multicentre, observational cohort of patients with *S. aureus* community-acquired pneumonia (CAP) requiring intensive care. All French adult and paediatric intensive care units (ICU) were contacted *via* the main learned medical societies (Société de Réanimation de Langue Française: ∼200 adult ICU; Groupe Francophone de Réanimation et d'Urgence Pédiatrique of the Société Française de Pédiatrie: 41 paediatric ICU) and invited to participate. Patients with *S. aureus* CAP (see criteria in supplementary methods) and admitted to a participating ICU were included between January 2011 and December 2016. Exclusion criteria were HIV-positive status, hospital-acquired pneumonia or admission to hospital in the past 3 months. Clinical, laboratory and therapeutic data were collected prospectively at admission to ICU and on day 1, 3 and 7. Severity was evaluated using age-adapted severity scores (see details in the supplementary methods).

Microbiology

The causative staphylococcal isolates were transmitted to the French National Reference Centre for Staphylococci for full characterisation and DNA array genotyping (supplementary methods) [17].

Statistical analysis

We determined whether the previous descriptions of different PVL-associated diseases, namely pleuropneumonia in children and staphylococcal necrotising pneumonia in adults correlated with identifiable age groups in patients with severe CAP. To this aim, patterns in patient age distribution were detected using a clustering procedure (supplementary figure S1). Following this, disease presentation and outcome in toddlers (age <3 years, as determined by the age clustering procedure) were compared to that found in older patients. The comparison of PVL-positive and PVL-negative cases was restricted to older patients (aged ⩾3 years) because 95% of toddlers had PVL-positive CAP. Between-group comparisons used Fisher's exact test or t-test, as appropriate. Possibly nonlinear changes of mortality rates in function of patient age were visualised using kernel density estimation with bootstrap confidence intervals (supplementary methods). Potential predictors of mortality were investigated using univariate and multivariable Cox proportional hazards models. To avoid bias due to row-wise deletion of cases containing missing data in multivariate models [18], all missing data were imputed prior to analysis using a nonlinear, random forest-based multiple imputation technique. Imputation uncertainty was accounted for by replacing binary factors (such as leukopenia, for example) by a probability estimate between 0 (absence) and 1 (presence).

To further examine the relationship between microbiological characteristics and outcome, we assessed whether the isolate's lineage and the presence of virulence factors, as determined using DNA arrays, predicted mortality and major clinical characteristics at admission, namely, rash, haemoptysis and leukopenia. These analyses were restricted to adult patients. All models included the Charlson score as a covariate to account for the diversity of age and baseline health in the cohort. Models with death as the response variable used Cox regression, with coefficients reported as hazard ratios. Models with rash, haemoptysis or leukopenia as the response variable used logistic regression, with coefficients reported as odds ratios. In a first series of models, each outcome was regressed on the clonal complex, treated as a categorical variable, to identify associations of the bacterial lineage with clinical presentation. Given the

strong genetic diversity in our collection, lineages with <10 isolates were pooled into an "other" category before regression analysis. In a second series of models, we examined a near-exhaustive array of virulence determinants for associations with death, rash, haemoptysis and leukopenia. A subset of the 332 loci and alleles, as determined using DNA arrays, was selected *a priori* based on their plausible role in virulence (*e.g.* excluding antimicrobial resistance determinants other than *mec*A) and nonredundancy (*e.g.* excluding the *luk*F component of *pvl* which is virtually always present with the *luk*S component; supplementary table S8). The alleles defining *agr* groups I to IV were also included, but not the lineage (clonal complex) which would be redundant with the genotypic profile. Variables present or absent in <10 isolates were excluded. A final set of 28 nonconstant genotypic determinants were included in the final analyses (supplementary table S8). Determinants were coded as 0 when absent and 1 when present. Ambiguous DNA array results (missing data, 0.8% of results) were imputed using the random forest procedure described earlier. Given the large number of determinants, we used sparse regression (least absolute shrinkage and selection operator (LASSO)) as implemented in the *glmnet* R package, to identify the determinants retained in sparse models [19]. Briefly, LASSO models are built sequentially by modifying a L1 penalisation constraint such that more penalised models include fewer predictors. The best-fitting models (using death, rash, haemoptysis or leukopenia as the response variable) were selected using leave-one-out cross-validation.

Analyses were conducted using R software v3.6.0 (www.R-project.org/). Anonymised data and software code required to reproduce the results are available at github.com/rasigadelab/severecap.

Results

Clustering analysis of patient age identifies specific patterns in PVL-positive and PVL-negative S. aureus *pneumonia*

A total of 228 patients were eligible. After removal of those who did not comply with the inclusion criteria, those who did not provide consent and those with missing case report forms, 163 patients were included (supplementary figure S1). The included patients were hospitalised in 77 ICUs and aged from 1 month to 87 years. 85 patients (52.1%) were infected by a PVL-positive *S. aureus*. Clustering by age of patients with PVL-positive CAP identified two nonoverlapping clusters aged <3 years and ⩾3 years (supplementary figure S2). In patients with PVL-negative CAP, we identified two overlapping clusters centred at ages 28 years and 60 years. Almost all cases in patients aged <3 years (hereafter "toddlers") were PVL-positive, while PVL positivity in older patients was evenly distributed according to age (supplementary figure S2). In contrast, PVL-negative CAP was exceptional in younger patients (only one case aged <15 years), and its prevalence increased with patient age, peaking at 60 years. Hence, toddlers accounted for 12.3% of all severe staphylococcal CAP in our cohort, and 22.3% of PVL-positive cases. The microbiological and clinical features of CAP differed markedly between toddlers and older patients (supplementary table S1). PVL positivity was more frequent in toddlers, more strongly so than methicillin resistance (seven out of 20 *versus* 23 out of 143; OR 2.8, 95% CI 0.85–8.6). Additional details on the specificities observed in toddlers are given in the supplementary results.

Mortality according to age

Mortality increased with age, reaching a plateau at ∼30 years in PVL-positive cases (figure 1). Age-dependent increase of mortality was more linear in PVL-negative cases (supplementary figure S3). Mortality was lower in toddlers than older patients (three out of 20 *versus* 52 out of 143; OR 0.31, 95% CI 0.06–1.15). In toddlers with PVL-positive CAP (supplementary figure S3c and d), most cases occurred before 12 months of age and were nonlethal, while mortality increased after 1 year of age.

Since only one toddler had PVL-negative CAP, we could not compare the outcomes of PVL-positive and -negative CAP in this group. Further comparisons according to PVL positivity and analysis of severity factors were restricted to patients aged ⩾3 years to avoid the interpretation bias that would have resulted from the pooling of such markedly different patients (namely, toddlers and older patients) in a single analysis.

PVL is associated with specific symptoms and is a severity factor in patients aged ⩾3 years

Among the 143 patients aged ⩾3 years, 66 (46.2%) had PVL-positive CAP. Compared to PVL-negative CAP, patients with PVL-positive CAP were younger and less likely to have an underlying condition (table 1). At admission, they had more frequent airway haemorrhage and cutaneous eruption or rash evoking immune system reaction. In addition, PVL-positive CAP patients were more likely to present with septic shock, leukopenia, elevated blood lactate and Sequential Organ Failure Assessment (SOFA) severity score, and to require extracorporeal membrane oxygenation (ECMO).

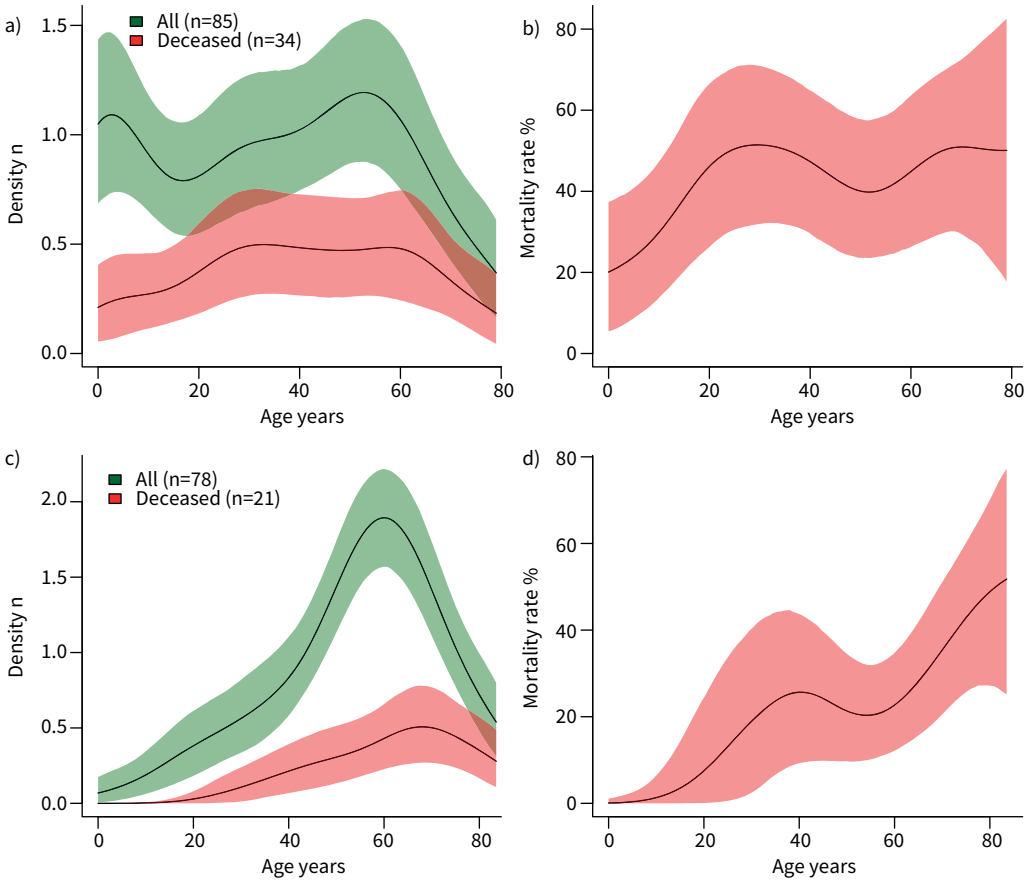

FIGURE 1 Mortality of patients with *Staphylococcus aureus* community-acquired pneumonia (CAP) according to age and the presence of the Panton–Valentine leukocidin (PVL). a, c) Density estimates of the occurrence of pneumonia in all patients of the considered group and in deceased patients. b, d) Estimates of mortality rate. Shaded areas present bootstrap-based 95% confidence bands of the estimates. Analyses were conducted separately for patients with a, b) PVL-positive CAP and c, d) PVL-negative CAP.

Appropriate initial antibiotic treatment, defined as one or more drugs active against the corresponding strain, was equally likely in PVL-positive and -negative CAP. However, the initial treatment was less frequently appropriate in methicillin-resistant *S. aureus* (MRSA) cases (OR 0.027, 95% CI 0.006–0.097). See the supplementary results for additional information on antibiotic treatment.

Most of the differences observed between patients with PVL-positive and -negative CAP at admission persisted during the first week in ICU (tables 2 and 3). The higher SOFA score at admission persisted at day 1, as did the higher median blood lactate. Temperature >39°C, airway haemorrhage and rash remained significantly more common in PVL-positive cases, who received more frequently inhaled nitric oxide and required ECMO support.

Survival

We used survival analysis to examine the influence of microbiological and clinical factors on lethal outcome in the 143 patients aged ⩾3 years. Kaplan–Meier survival curves suggested that PVL-positivity and methicillin resistance contributed additively to mortality (figure 2); median (interquartile range (IQR)) survival time was 1 day (0–7 days) for PVL-positive cases and 7 days (3–14 days) for PVL-negative cases (p=0.02, Mann–Whitney U-test). Among the 91 survivors, length of ICU stay was longer in those with PVL-positive CAP (median, IQR time until discharge 39 days, 26–68 days) than in those with PVL-negative CAP (29 days, 20–42 days; p=0.01, Mann–Whitney U-test).

We then used bivariate and multivariable Cox proportional hazards models to identify potential independent risk factors for mortality (table 4). Predictors were included in the models based on their

TABLE 1 Clinical and laboratory characteristics at admission according to Panton–Valentine leukocidin (PVL) status in patients aged ⩾3 years

	PVL-positive *S. aureus*		PVL-negative *S. aureus*		p-value
	Patients	Missing	Patients	Missing	
Patients	66		77		
Delay in days between onset and ICU admission	3.39±3.74	0	4.64±4.80	4	0.092
Female/male	31/35	0	27/50	0	0.173
Age years	47 (28.7–60.2)	0	58 (48–67)	0	<0.001
Children age <18 years	6 (9.1)	0	1 (1)	0	0.049
MRSA	14 (21.2)	0	9 (11.7)	0	0.170
Absence of underlying condition[#]	39 (59.1)	0	22 (28.6)	0	<0.001
Charlson's score	1.18±0.18	0	2.14±0.22	0	0.001
Temperature >39°C	35 (53)	0	29 (38.2)	1	0.092
Airway haemorrhage	29 (43.9)	0	18 (23.4)	0	0.012
Toxin-mediated rash	14 (21.2)	0	5 (6.6)	1	0.013
Bilateral pneumonia	46 (70.8)	1	52 (68.4)	1	0.855
Significant pleural effusion[¶]	3 (4.5)	0	1 (1.4)	3	0.343
Leukocytes $G \cdot L^{-1}$	4.60 (1.4–15)	1	9.34 (5–15.8)	3	0.011
Leukopenia <3 $G \cdot L^{-1}$	28 (43.1)	1	14 (18.9)	3	0.003
Blood lactate $mg \cdot L^{-1}$	3.95 (2.3–5.5)	8	2.4 (1.7–4.6)	9	0.015
CRP $mg \cdot L^{-1}$	327.5 (191.2–396.7)	26[+]	251.6 (127–351)	18[+]	0.041
Procalcitonin $\mu g \cdot L^{-1}$	137.5 (51.7–488.5)	40[+]	57 (8–200)	58[+]	0.032
Appropriate antibiotics	47 (75.8)	4	62 (80.5)	0	0.539

Data are presented as n, mean±SD, median (interquartile range) or n (%), unless otherwise stated. Percentages are calculated among those with data. *S. aureus*: *Staphylococcus aureus*; ICU: intensive care unit; MRSA: methicillin-resistant *S. aureus*; CRP: C-reactive protein. [#]: underlying conditions include malignancies, tobacco smoking, alcohol abuse, diabetes and body mass index >30 $kg \cdot m^{-2}$; [¶]: needing drainage; [+]: inflammatory markers (CRP or procalcitonin) are missing for 14 patients (seven in each group).

expected relevance to disease outcome. In addition to PVL and methicillin resistance, these included baseline patient characteristics, namely sex and the Charlson comorbidity score; characteristics reflecting 1) severity upon admission, namely the SOFA score, haemoptysis, leukopenia and blood lactates, 2) inflammatory reaction, namely rash and blood procalcitonin and 3) a risk factor for staphylococcal superinfection of the lung, namely a flu-like illness; and the appropriateness and expected toxin-suppressing activity of the antibiotics received (see the supplementary results for details). In the best-fitting multivariable model, the independent predictors of death were methicillin resistance, haemoptysis, rash, leukopenia, elevated blood lactates and the absence of a flu-like illness (supplementary table S3).

TABLE 2 Clinical and laboratory characteristics during stay according to Panton–Valentine leukocidin (PVL) status in patients aged ⩾3 years

	PVL-positive *S. aureus*		PVL-negative *S. aureus*		p-value
	Patients	Missing	Patients	Missing	
Patients	66		77		
Temperature >39°C	44 (75.9)	8	44 (58.7)	2	0.043
Airway haemorrhage	35 (59.3)	7	28 (37.3)	2	0.015
Toxin-mediated rash	15 (25.4)	7	8 (10.7)	2	0.037
Bilateral pneumonia	45 (78.9)	9	61 (83.6)	4	0.505
Significant pleural effusion[#]	10 (16.4)	5	10 (13.7)	4	0.808
Highest CRP $mg \cdot L^{-1}$	352 (230–447)	38	228 (106.0–347)	38	0.017
Highest procalcitonin $\mu g \cdot L^{-1}$	91 (28.5–375)	37	35.4 (3.3–98.6)	47	0.032

Data are presented as n, n (%) or median (interquartile range), unless otherwise stated. Percentages are calculated among those with data. *S. aureus*: *Staphylococcus aureus*; CRP: C-reactive protein. [#]: needing drainage.

TABLE 3 Severity scores and markers at admission and during evolution according to Panton–Valentine leukocidin (PVL) status in patients aged ⩾3 years

	PVL-positive *S. aureus*		PVL-negative *S. aureus*		p-value
	Patients	Missing	Patients	Missing	
Patients	66		77		
Admission					
SOFA score	8.9±5.68	2	6.9±5.01	8	0.004
Septic shock	31 (48.4)	4	22 (30.1)	2	0.035
ARDS	20 (32.8)	7	16 (22.9)	5	0.352
Severe ARDS	12 (19.7)	7	10 (14.3)	5	0.485
Invasive ventilation	32 (48.5)	0	31 (42.5)	4	0.499
ECMO	11 (16.9)	1	3 (4.2)	5	0.022
Day 1					
SOFA score	10.9±5.96	14	8.8±5.25	8	0.040
Septic shock	31 (54.4)	9	28 (40.6)	8	0.152
ARDS	27 (50)	12	32 (50)	13	1
Severe ARDS	21 (38.9)	12	19 (29.7)	13	0.332
Death	7 (10.6)	0	4 (5.2)	0	<0.001
Lactate variation[#]	0.62±4.38	19	−0.69±2.10	14	0.063
Day 3					
SOFA score	10.6±6.51	24	8.1±5.34	8	0.045
Cumulative death[¶]	17 (25.8)	0	7 (9.1)	0	<0.001
Day 7					
SOFA score	8±6.84	29	6.8±5.70	16	0.347
Cumulative death[¶]	23 (34.8)	0	10 (13)	0	<0.001
During whole ICU stay					
Invasive ventilation	56 (84.8)	0	60 (81.1)	3	0.655
Inhaled NO	21 (33.3)	3	11 (15.3)	5	0.016
ECMO	20 (32.3)	4	8 (11)	4	0.003
Death	31 (47.0)	0	21 (27.3)	0	0.023

Data are presented as n, n (%) or median (interquartile range), unless otherwise stated. Percentages are calculated among those with data. *S. aureus*: *Staphylococcus aureus*; SOFA: Sequential Organ Failure Assessment; ARDS: acute respiratory distress syndrome; ECMO: extracorporeal membrane oxygenation; ICU: intensive care unit. [#]: between day 0 and day 1; [¶]: from day 0 to day 3 or day 7.

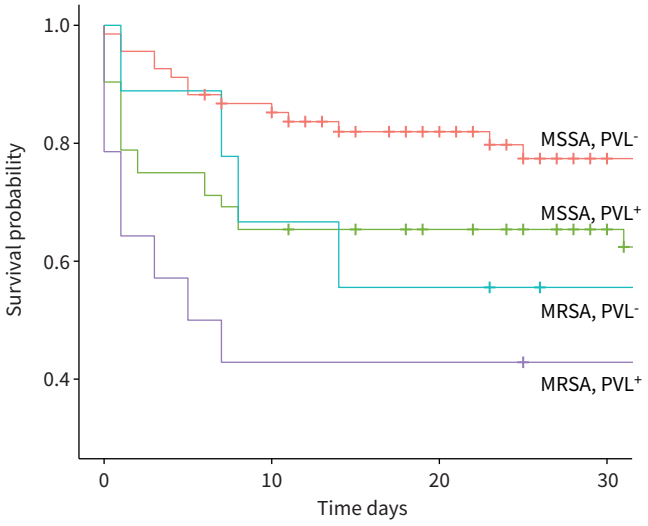

FIGURE 2 Survival of 143 patients aged ⩾3 years with *Staphylococcus aureus* pneumonia. Survival after intensive care unit admission according to whether the causative strain was methicillin-resistant (MRSA) or -susceptible (MSSA) and whether it harboured the Panton–Valentine leukocidin (PVL) toxin is shown. +: censored (discharged) patients. Log-rank test, p=0.0007.

TABLE 4 Cox regression analysis of predictors of death in patients aged ⩾3 years with *Staphylococcus aureus* pneumonia, including clinical predictors at admission and microbiological predictors

	Bivariate models	Full model	Best-fitting model
Charlson comorbidity score (per point)	1.05 (0.91–1.21)	1.03 (0.85–1.26)	
Male sex	0.68 (0.39–1.17)	0.95 (0.51–1.78)	
PVL	1.95 (1.12–3.41)	0.87 (0.42–1.83)	
Methicillin resistance	2.57 (1.41–4.69)	3.86 (1.34–11.16)	2.87 (1.53–5.40)
SOFA score	1.11 (1.05–1.16)	1.03 (0.96–1.11)	
Flu-like illness	0.71 (0.41–1.23)	0.32 (0.15–0.68)	0.32 (0.17–0.60)
Haemoptysis	2.62 (1.52–4.52)	1.87 (0.98–3.54)	2.15 (1.17–3.93)
Rash	2.17 (1.12–4.23)	2.59 (1.22–5.48)	2.52 (1.24–5.12)
Leukopenia <3 G·L^{-1}	3.45 (2.00–5.97)	2.38 (1.19–4.76)	2.38 (1.23–4.58)
Procalcitonin per two-fold increase	1.40 (1.21–1.63)	1.05 (0.88–1.24)	
Lactates per two-fold increase	2.40 (1.85–3.12)	2.23 (1.52–3.28)	2.64 (1.95–3.57)
Adapted antimicrobial therapy	0.82 (0.44–1.53)	1.54 (0.52–4.59)	
Antitoxin therapy	2.67 (1.48–4.81)	1.29 (0.63–2.65)	

Data are presented as hazard ratio (95% CI). Bivariate models were independent Cox proportional hazards models, one per predictor. The full multivariable model included all predictors (likelihood ratio test, p<0.001; Akaike information criterion (AIC) 420 with 13 degrees of freedom). The best-fitting model was obtained using a stepwise procedure, starting from the full model, and minimising the AIC (409 with 6 degrees of freedom). Confidence interval widths were not corrected for test multiplicity. PVL: Panton–Valentine leucocidin; SOFA: Sequential Organ Failure Assessment.

Interestingly, PVL positivity significantly predicted death in bivariate analysis (table 4) while the best-fitting multivariable model included previously recognised severity factors in PVL-positive lung infection, namely haemoptysis and leukopenia [20], but not PVL itself. The exclusion of PVL, but not its clinical consequences from the final model is suggestive of a chain of consequence between PVL, severity factors and death, where the severity factors mediate the effect of PVL on death [21]. To verify this interpretation, we constructed an additional multivariable Cox regression model including only microbiological and baseline predictors, but not the potential consequences of PVL such as treatment or severity factors. In this model, PVL positivity predicted death (adjusted hazard ratio (HR) 1.81, 95% CI 1.04–3.17) independent of methicillin resistance, sex, Charlson score and flu-like illness. Thus, PVL positivity was a risk factor of mortality independent of other baseline factors. See the supplementary results and supplementary figure S4 for additional data on severity-associated features and on interactions between predictors of mortality.

Contribution of the strain genetic background and other virulence factors to severity

Although PVL and methicillin resistance have been repeatedly associated with poor outcome in previous studies, it is still debated whether they exert a direct effect on outcome or rather act as surrogate markers for more virulent lineages. To examine this issue, we tested whether mortality and major severity factors at admission, namely, rash, haemoptysis and leukopenia, could be predicted by the isolate's lineage (supplementary table S2) and virulence gene content, independently of the Charlson comorbidity score.

In models using lineage as a predictor (table 5), the Charlson score predicted leukopenia, but not death, rash or haemoptysis, while the isolate's lineage contributed to predict rash, possibly leukopenia (p=0.06, analysis of deviance Chi-squared test), but not death or haemoptysis. Substantial associations between lineage and outcome were found, namely between CC121 and a rash, and between CC152, CC398 and leukopenia (table 5). In an additional, mixed-effect Cox regression including one random intercept per lineage, both *mec*A (HR 2.25, 95% CI 1.18–4.27) and *pvl* (HR 1.98, 95% CI 1.08–3.62) were independent predictors of death. Collectively, these results indicate that the *S. aureus* lineage *per se* does not predict death independently of *mec*A and *pvl*, which favours a direct role of these determinants rather than an indirect role as lineage markers. However, other clinical characteristics such as rash or leukopenia were influenced by the strain's lineage, suggesting a potential role of other virulence factors.

To further decipher the relationships between virulence and CAP outcome, we examined 28 virulence determinants for associations with death, rash, haemoptysis and leukopenia (see supplementary figure S5 for correlation between the selected predictors). The best-fitting LASSO regression models for each outcome, selected using leave-one-out cross-validation, are reported in table 6 (see supplementary figure

TABLE 5 Association of *Staphylococcus aureus* lineage with death, rash, haemoptysis and leukopenia in 143 adult patients with severe community-acquired pneumonia

	Regression coefficient			
	Death HR (95% CI)	Rash OR (95% CI)	Haemoptysis OR (95% CI)	Leukopenia OR (95% CI)
Charlson comorbidity score	1.11 (0.95–1.29)	0.90 (0.61–1.32)	0.97 (0.78–1.21)	0.80 (0.62–1.04)
CC121	1.71 (0.66–4.42)	24.19 (4.85–120.48)	0.68 (0.16–2.90)	0.90 (0.16–4.97)
CC152	2.55 (1.07–6.09)	0.47 (0.05–4.61)	3.30 (0.96–11.29)	7.84 (2.06–29.81)
CC30	0.69 (0.25–1.89)	0.87 (0.15–4.95)	0.68 (0.21–2.23)	1.17 (0.31–4.40)
CC398	1.07 (0.39–2.94)	0.62 (0.07–5.62)	1.04 (0.30–3.58)	4.47 (1.22–16.34)
CC5	0.38 (0.09–1.66)	[¶]	0.56 (0.14–2.33)	0.79 (0.15–4.31)
CC8	1.03 (0.34–3.11)	[¶]	0.53 (0.10–2.88)	2.62 (0.52–13.15)
CC80	1.59 (0.58–4.35)	0.94 (0.10–9.13)	1.39 (0.34–5.68)	3.47 (0.77–15.58)
Charlson comorbidity score[#]	p=0.54	p=0.45	p=0.32	p=0.02
Lineage[#] (CC)	p=0.15	p<0.001	p=0.35	p=0.01

Lineages with sample size <10 were pooled and used as the reference category. Death prediction model used multivariable Cox regression, with coefficients reported as hazard ratios (HRs). Other models used multivariable logistic regression, with coefficients reported as odds ratios. [#]: analysis of deviance, Chi-squared test; [¶]: unresolved coefficient due to small sample size.

S5 and supplementary tables S4 to S7 for details of LASSO models). This analysis confirmed that both *mec*A and *pvl* were independent predictors of death, even when all other virulence markers were considered. In addition, the *pvl* was an independent predictor of haemoptysis and leukopenia, but not rash. Rash was mainly predicted by an *agr*IV background, in line with the association of rash with lineage CC121 of the *agr*IV background (table 5). Of note, PVL remained among the top-ranking predictors not included in the best-fitting model (supplementary table S5).

The LASSO models of haemoptysis and leukopenia yielded additional insights into the interplay of virulence factors with CAP presentation. In addition to PVL, *edin*B (encoding the epidermal differentiation

TABLE 6 Association of *Staphylococcus aureus* virulence determinants with death, rash, haemoptysis and leukopenia in 143 adult patients with severe community acquired pneumonia

	Coefficient in best-fitting LASSO model			
	Death HR	Rash OR	Haemoptysis OR	Leukopenia OR
Charlson score (per point)	–	–	–	0.87
***mec*A**	1.89	–	–	–
***agr*I**	–	–	–	1.52
***agr*II**	–	–	–	0.79
***agr*IV**	–	13.99	–	–
***luk*S-PV**	1.23	–	1.15	2.52
***edin*B**	–	–	2.27	1.60
cap5	–	*0.63*	–	*1.78*
etD	–	–	–	*2.32*
sea	–	–	–	2.05
egc	–	–	–	0.92
***luk*D**	–	–	–	0.53
***ica*C**	0.81	–	–	–
cna	–	1.31	–	–
eap	0.86	–	–	–
***sdr*D**	0.99	–	–	–

Models with least absolute shrinkage and selection operator (LASSO) (L1) penalty based on Cox (death) or logistic (rash, haemoptysis, leukopenia) regression. The best-fitting model minimises prediction error in leave-one-out cross-validation. In all models, 49 nonconstant virulence factors as well as the Charlson comorbidity score were considered as potential predictors. Note that LASSO models do not define confidence intervals. HR: hazard ratio; –: predictor not included in model.

inhibitor) was associated with both conditions, and the staphylococcal enterotoxin A gene (*seA*) gene predicted leukopenia.

Discussion

The present results improve our understanding of the role of PVL in different clinical presentation and severity of staphylococcal pneumonia. In addition, we identify the role of other severity-associated factors including bacteriological ones, and their link with PVL.

PVL-positive CAP and its severity were not evenly distributed according to age. PVL-negative CAP was virtually absent in toddlers and mortality was low. Many infectious processes vary in terms of presentation between young and older subjects, but the differences we observed exceed the expected variations due to age alone. For instance, radiological findings in pneumonia are usually similar at all ages [22]; however, we observed substantial radiological differences, notably regarding pleural effusion, pneumothorax and unilateral involvement that were all more frequent in toddlers. In addition, it was unexpected to observe a lower mortality in very young children compared to young adults without underlying conditions. The lower severity associated with PVL in toddlers could be due to 1) immunological immaturity, considering that local inflammation plays a central role in PVL-induced lesions [23]; 2) passive protection by maternal antibodies; or 3) the route of infection (the bullous lesions in toddlers point towards the involvement of the supporting tissue), suggesting a haematogenous route of infection as opposed to a probable inhalation route in adolescents and adults leading to direct necrosis of the respiratory epithelium. The observed specific symptoms and lower severity in toddlers might reflect a combination of one or more of the factors described, but also other features yet to be identified (see additional comments in the supplementary material). Thus, despite similarities in terms of site of infection and bacteriology, staphylococcal CAP in young children (*i.e.* pleuropneumonia) and staphylococcal necrotising pneumonia in adults should be considered as two distinct entities.

As we uncovered major differences according to age in PVL-positive staphylococcal pneumonia, the lack of consideration of age-specificity of the disease severity may at least partially explain some conflicting results reported in the literature [14, 24]. Considering that PVL-negative staphylococcal pneumonia is almost absent in the youngest patients, toddlers should be excluded from analysis to assess the role of PVL in severity.

We observed that PVL was associated with specific symptoms in staphylococcal pneumonia of adolescents and adults. Previously described features strongly associated with PVL [2, 7] were observed herein among patients aged ⩾3 years: PVL-positive *S. aureus* pneumonia occurred mainly in younger people without underlying conditions and was associated with cutaneous rash, airway haemorrhage and leukopenia. However, logistic regression revealed that rash was most strongly predicted by the *agrI*V-CC121 genetic background, independently of PVL or the presence of superantigens. The basis of the association of PVL with rash appears elusive, although rash as a symptom remains a relevant marker of PVL-positive CAP.

Airway haemorrhage is a consequence of respiratory epithelial necrosis, as described in lung autopsies [1, 2]. Haemorrhage is an indirect effect of PVL, since the presence of polymorphonuclear (PMN) cells in the lung is required for PVL-induced necrotic lesions of the epithelium [25]. PMN cell influx to the lung may be generated by two nonexclusive mechanisms: a preceding viral infection producing chemoattractant for neutrophils by epithelial cells [25], and PVL-mediated inflammasome activation (releasing interleukin (IL)-1β), leading to PMN cell recruitment and activation [26]. Interestingly, this inflammatory pathway is amplified by the release of pathogen- and damage-associated molecular patterns from dying neutrophils, possibly explaining the fulminant nature of PVL-associated CAP [27]. Of note, the PSMα3-, β-, γ-, δ-haemolysins and LukDE have been shown to synergise with PVL to amplify IL-1β release, indicating that these factors cooperate with PVL to trigger inflammation [26]. However, among the factors listed above, LukDE is the only variable genotypic determinant and there was no direct association with haemoptysis in the LASSO model. Conversely, the LASSO model revealed that beside PVL, *edin*B was the second genotypic marker associated with haemoptysis, a feature not described previously. Edin-B elicits large transcellular tunnels in endothelial cells (macroapertures) inducing a loss of barrier function and providing direct access of the endothelium basement membrane to *S. aureus*. In mouse models, Edin-B promotes the translocation of *S. aureus* to the bloodstream during the course of pneumonia [28]. How these properties translate into haemoptysis remains to be explored.

We show that leukopenia is another major feature associated with PVL and death. The relationship between leukopenia and PVL has a mechanistic explanation since the identification of C5aR on myeloid cells as the receptor for lukS-PV [29]. Beside PVL, other determinants contributed to leukopenia, such as CC152 and CC398 lineage, Edin-B and SEA. This later association is novel in humans; it potentially

results from extravasation of cells in a Vβ-unrestricted manner as demonstrated experimentally in rabbits for another major superantigen, the TSST-1 [30].

One objective of the present study was to assess the link between PVL and severity, considering that such an association has been controversial since the first description of PVL-associated pneumonia in 2002 [2]. The frequency of PVL in severe CAP in adults herein contrasts with the low frequency of PVL (<5%) in the staphylococcal carriage population in France [17], and this striking difference constitutes the initial evidence for an association of PVL with severity. We observed that PVL, especially but not exclusively when associated with methicillin resistance, was an independent factor of mortality in staphylococcal CAP even when other virulence markers were considered, outweighing protective factors such as young age and the absence of underlying conditions. The present study indicates that PVL is associated with nonspecific severity markers of infection such as elevated lactate or presence of septic shock at admission and absence of reduction in lactate between admission and day 1. All these factors are associated with a higher mortality in sepsis, irrespective of the bacteria involved [31, 32], and their higher frequency in the PVL-positive cases confirms greater severity in such patients. Surrogate markers of respiratory failure, such as the need for nitric oxide and for ECMO, were more frequent in PVL-positive cases, which also indicates greater severity in accordance with recent studies [33, 34]. The major symptoms associated with PVL (*i.e.* airway haemorrhage and leukopenia) were associated with mortality in the multivariable prediction model (table 4), strongly suggesting that these factors mediate the link between PVL and death.

Since its first description [2], PVL-positive *S. aureus* pneumonia has been reported worldwide [34–37]. A particular situation is observed in the United States where the description of necrotising pneumonia coincided with the emergence of the PVL-positive CA-MRSA USA300 lineage [4, 38]. PVL has been also associated with the CA-MRSA ST-80 lineage in Europe [39]. However, only 11 and five strains of the present study belong to these clones, respectively, and PVL was distributed within a large diversity of genetic backgrounds (*i.e.* 13 CCs) with a majority of methicillin-susceptible *S. aureus*, ruling out a possible clonal bias associated with PVL-positive cases. The lack of genetic diversity of CA-MRSA in the US may have initially led to confusion in the understanding of the determinant of severity associated with staphylococcal CAP. Thus, methicillin resistance was thought to be the prominent determinant for severity, presumably by inducing a delay in initiation of appropriate antibiotics, in line with our observations. However, this higher mortality in MRSA was present both in PVL-positive and PVL-negative pneumonia patients.

Mortality increased at ages >60 years (markedly in PVL-negative cases), which is anticipated for bacterial pneumonia; however, mortality reached a plateau in PVL-positive cases at ∼30 years, an observation for which we have only hypothetical and nonexhaustive explanations. Mortality results from a balance of protective and deleterious factors. Protection is probably conferred by a gradual acquisition of toxin neutralising and opsonising antibodies against *S. aureus*. Conversely, enhanced severity may be caused by a highly reactive innate immune system (typically lacking in toddlers). Alternatively, the age-dependent acquisition of autoantibodies against cytokines and chemokines involved in *S. aureus* response (IL-1β, tumour necrosis factor-α, macrophage inflammatory protein-1α, granulocyte–macrophage colony-stimulating factor, IL-17A and interferon (IFN)-γ [40]) may impair protective immune responses in a subset of young adults, mimicking the immunosuppression associated with autoantibodies against type I IFNs in patients with severe severe acute respiratory syndrome coronavirus 2 [41].

We acknowledge limitations of the present study, most notably the observational design, whereby all participating centres were encouraged to enrol patients fulfilling the inclusion criteria. We cannot exclude under-reporting, and a higher reporting of the most severe cases may have occurred, but this is unlikely to have affected directly the comparison between PVL-positive and PVL-negative cases. The number of missing data were limited and exhaustivity was reached for all major parameters. Furthermore, the study was restricted to France, but covers the French territory almost entirely with 77 participating centres, thus limiting possible local epidemiological bias.

In conclusion, the present study demonstrates the association of PVL with two distinct facets of staphylococcal CAP with marked differences between toddlers and adolescents/adults regarding clinical presentation and outcome. In toddlers, PVL appears to be prominent in staphylococcal pneumonia, presentation matches with pleuropneumonia, and standard of care in modern ICU appears to be sufficient for favourable outcome. In contrast, PVL-positive CAP in adolescents and adults remains extremely severe despite aggressive management; it deserves further research to develop tailored therapeutic approaches.

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