

# Blood culture fluorescence rates predict severity and mortality of invasive pneumococcal pneumonia

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**Abstract** Invasive pneumococcal pneumonia is associated with high rates of mortality. Clinical assessment tools have poor sensitivity for predicting clinical outcomes. Molecular measurements of bacterial load correlate closely with clinical outcome but require specialist facilities and expertise. This study describes how routine blood culture testing can estimate bacterial load and predict clinical outcome for invasive pneumococcal pneumonia. Between December 2009 to March 2014, clinical and laboratory data were collected for 50 patients with *Streptococcus pneumoniae* bacteraemia secondary to community-acquired pneumonia. Fluorescence rates (FR) were calculated from growth curves generated by BACTEC blood culture analysers by dividing change in fluorescence units (FU), measured at the first point of detectable fluorescence and at the point of automated BACTEC positivity, by time in hours. The mean age of the patients was 70.6 years (49.6–86.3). Forty patients survived invasive pneumococcal disease and ten patients died. These two groups did not significantly differ by demographic or clinical characteristics. The mean FR for the non-survival group ( $3.62 \times 10^{-3}$  FU/h) was significantly higher ( $p < 0.001$ ) than that of the survival group ( $1.73 \times 10^{-3}$  FU/h). FR did not vary by serotype. We determined that an FR of  $2.59 \times 10^{-3}$  FU/h might represent a useful threshold for predicting high mortality risk with a sensitivity of 91 % and

a specificity of 97 %. Our FR calculation uses cheap and accessible routine blood culture techniques to predict mortality in a small retrospective cohort study. In patients admitted to hospital with pneumococcal bacteraemia and, potentially, other organisms, this single tool could guide early escalation of clinical care.

## Introduction

*Streptococcus pneumoniae* (pneumococcus) is the main cause of community-acquired pneumonia worldwide [1]. Bacteraemia secondary to pneumococcal pneumonia is the most common presentation of invasive pneumococcal disease (IPD) and is associated with high mortality rates, particularly in elderly populations [2–7]. Mortality rates are highest during the first days of admission [8, 9]. During this critical period, precise diagnostic evaluation, combination antibiotic therapy [10, 11], adjunctive treatments [12] and early admission to the intensive care unit (ICU) [13] may all reduce inpatient mortality from IPD. The accurate identification of individual patients who could benefit from these interventions remains inexact and challenging. Prognostic scoring systems [such as the pneumonia severity index (PSI) [14] and the confusion, elevated blood urea nitrogen level, respiratory rate and blood pressure plus age  $\geq 65$  years (CURB 65 score) [15]] stratify patients in large cohorts according to mortality risk in order to identify those who can be safely managed in an outpatient setting. However, both of these clinical scores and consensus guidelines do not reliably identify individual patients requiring ICU escalation [13, 16–19]. In contrast, there are increasing data that correlate pneumococcal bacterial load measured by molecular assays with clinical outcome [20, 21]: detection of high bacterial load has specificity exceeding 80 % for septic shock and mechanical ventilation [21–23]. Thus, besides

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diagnostic utility in organism identification, the quantification of bacterial load may be useful in stratifying individuals with IPD to determine the site and intensity of inpatient care [13, 24]. DNA assays have increasing sensitivity and utility in the diagnosis of IPD in patients with negative blood cultures [21, 25]. However, DNA assays do have limitations: they may be only 60–85 % sensitive in patients with positive blood cultures, do not routinely yield antibiotic sensitivity data and require potentially expensive specialist facilities and expertise [21, 23]. Culture of viable pathogens from the bloodstream is intrinsically superior to the detection of pathogenic DNA and remains fundamental to the initial diagnostic work-up for sepsis [26]. The aim of this study was to determine the relationship of bacterial load to clinical outcome for patients with invasive pneumococcal pneumonia by using an affordable and sensitive method in a routine diagnostic microbiology laboratory.

## Methods

This was a retrospective study of adult patients admitted via the emergency department (ED) to hospital with invasive pneumococcal community-acquired pneumonia (CAP).

### Study population

During the study period (December 2009 to March 2014), patients were identified with *S. pneumoniae* bacteraemia secondary to CAP. CAP was defined as new or worsening cough, with or without sputum production, associated with an abnormal temperature ( $T < 35.6\text{ }^{\circ}\text{C}$  or  $> 37.8\text{ }^{\circ}\text{C}$ ) or abnormal serum inflammatory markers [leucocytosis, leucopaenia, elevated C-reactive protein (CRP)], or as new pulmonary infiltrates on chest radiography associated with an abnormal temperature or abnormal serum inflammatory markers. The hard-copy notes for a single patient were not available, despite the availability of laboratory and radiology records, and, thus, the antibiotic history and PSI score could not be recorded for that individual. Patients receiving antimicrobial therapy prior to blood cultures were excluded from the study.

The study cohort was organised into two groups: survived or died based on the mortality outcome of the pneumococcal CAP episode.

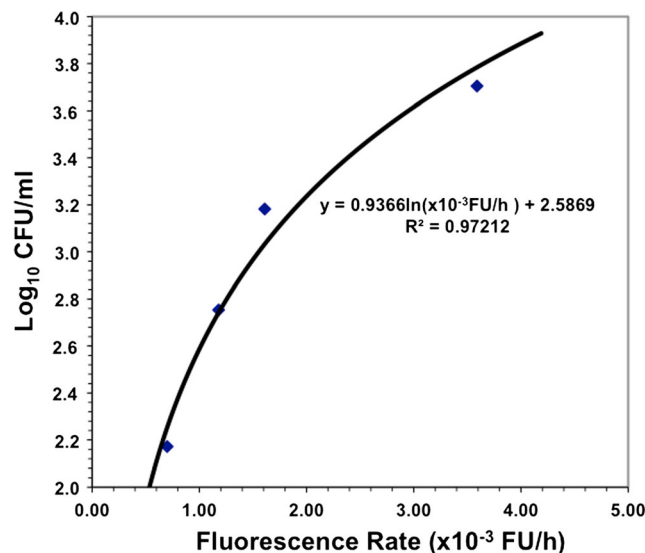
### Clinical data collection

A retrospective review of hard-copy and computer-based notes and letters, and pathology and radiology data was undertaken. Characteristics recorded were: demographics (age, gender, ethnicity), clinical characteristics [co-morbidities: immunocompromise, diabetes mellitus, chronic obstructive pulmonary disease (COPD), smoking history, chronic liver, heart, renal and neurological diseases, active malignancy,

human immunodeficiency virus (HIV) infection, steroid use], clinical outcomes at any time during admission [acute respiratory distress syndrome (ARDS), radiological progression, severity score according to the PSI [14], septic shock, acute kidney injury (AKI), ICU admission, intubation, non-invasive ventilation, video-assisted thoracoscopy (VATS) for empyema] and microbiological characteristics [sputum culture, endotracheal aspiration culture, bronchoalveolar lavage (BAL) culture, pleural fluid culture, pneumococcal urinary antigen].

### Clinical definitions

Comorbidity was defined as the presence of one of the following chronic conditions: immunocompromise (defined as primary immunodeficiency or secondary immunodeficiency due to splenectomy, haematological malignancy, autoimmune disorder, chemotherapy or radiotherapy within 4 weeks prior to admission), diabetes mellitus (defined as receiving any oral or subcutaneous therapy for diabetes mellitus), COPD (defined as the presence of airflow limitation due to chronic bronchitis or emphysema), current smoker (any number of cigarettes/day), heart disease (defined as prior elective or emergency percutaneous coronary intervention or coronary artery bypass grafting, or New York Heart Association classes III or IV symptoms of heart failure), liver disease (defined as biopsy-proven cirrhosis or past medical history of complications of portal hypertension, including upper gastro-intestinal bleeding, encephalopathy, spontaneous bacterial peritonitis or more



**Fig. 1** The standard curve for *Streptococcus pneumoniae* (NCTC 12977 strain) was generated by plotting the mean of at least four samples of each ten-fold dilution ( $\text{log}_{10}$  CFU/ml) against the corresponding fluorescence rate (FR). The generated equation of the logarithmic regression curve,  $y = 0.9366\ln(\times 10^{-3}\text{FU/h}) + 2.5869$ , was used to estimate the bacterial load in all blood culture samples

than one episode of paracentesis), chronic kidney disease [CKD stages II to V inclusive defined by the Renal Association UK as estimated glomerular filtration rate (eGFR) less than 90 ml/min/1.73 m<sup>2</sup> on two serum samples at least 90 days apart [27]], neurological condition (defined as any documented neurological condition, including dementia, on the initial medical clerking or available clinic letters),

active malignancy, HIV, corticosteroid use (equivalent of 20 mg prednisolone or more for at least 2 weeks prior to admission). ARDS diagnosed according to the criteria of the American–European Consensus Conference Committee [28]. Rapid radiological progression was defined as an increase in the size of pulmonary infiltrates of chest radiography by 50 % or more at 48 h after presentation. Septic shock was defined as

**Table 1** Clinical and microbiological characteristics of patients organised into three cohorts: overall, died and survived<sup>a</sup>

Patient characteristics	Overall	Died	Survived	<i>p</i> -Value
<b>Demographics</b>				
Total number	50	10	40	
Mean age, years (range)	70.6 (49.6–86.3)	80.9 (40.8–92.0)	66.4 (49.6–84.5)	0.194
Male	19 (38)	5	14	0.564
Female	31 (62)	6	25	
White ethnicity	35 (70)	8	27	0.823
<b>Clinical features</b>				
Immunocompromise	7 (14)	2	5	0.651
Diabetes mellitus	6 (12)	2	4	0.475
Current smoker [22] <sup>b</sup>	11 (41)	2	9	0.97
COPD	9 (18)	1	8	0.384
Liver disease	4 (8)	2	2	0.159
Heart disease	2 (4)	1	1	0.329
CKD	5 (10)	0	5	0.211
Neurological condition	8 (16)	3	5	0.248
Active malignancy	8 (16)	1	7	0.479
HIV [41]	1 (11)	0	1	0.708
Steroid use	2 (4)	0	2	0.443
Any comorbidity	30 (60)	7	23	0.78
<b>Clinical outcomes</b>				
ARDS	4 (8)	2	2	0.159
Rapid radiological progression [27]	6 (26)	3	3	0.002
Mean PSI score [1] (±SD)	116.1 (±49.3)	155.5 (±46.2)	104.7 (±44.5)	0.002
<b>Mean PSI class [1]</b>				
PSI I	2 (4)	0 (0)	2 (5)	0.209
PSI II	9 (18)	1 (10)	8 (20)	
PSI III	5 (10)	0 (0)	5 (12.5)	
PSI IV	12 (25)	2 (20)	10 (25)	
PSI V	21 (43)	8 (80)	13 (32.5)	
Septic shock	7 (14)	3	4	0.151
AKI [1]	12 (24)	7 (70)	5 (12.5)	<0.001
ICU admission	9 (18)	3 (30)	6 (15)	0.365
Intubation	2 (4)	2 (20)	0 (0)	0.007
NIV [5]	1 (2)	0 (0)	1 (2.5)	0.613
VATS	4 (8)	0 (0)	4 (10)	0.268
<b>Microbiological characteristics</b>				
FR (×10 <sup>-3</sup> FU/h; IQR)	–	3.62 (2.99–4.39)	1.73 (1.22–2.04)	<0.001
TTD (h; IQR)	–	9.79 (5.56–14.02)	10.76 (6.69–14.83)	0.247

<sup>a</sup> Percentages in parentheses (%) calculated for the total number of patients in each cohort

<sup>b</sup> Total number of patients with missing data represented in square parentheses, [total number]

systolic blood pressure of 90 mmHg despite fluid resuscitation of approximately 20 ml/kg. AKI was defined by the Renal Association UK as serum creatinine rise by  $\geq 26 \mu\text{mol/L}$  within 48 h or  $\geq 1.5$ -fold from the reference value, or urine output  $< 0.5 \text{ ml/kg/h}$  for  $> 6$  consecutive hours [29].

### Blood sample collection and processing

Whole blood samples (8–10 ml) were collected from the patients and inoculated into each vial of the blood culture set, which includes an aerobic bottle (BD BACTEC Plus Aerobic/F Medium) and an anaerobic bottle (BD BACTEC Lytic/10 Anaerobic/F), according to the hospital policy for taking blood samples for blood culture. Blood culture vials were incubated into the BACTEC blood culture analyser (BD BACTEC FX) within 2 h of taking blood samples.

### Detection of *S. pneumoniae* and quantification of the fluorescence rate of bacterial growth

Bacterial identification and susceptibility testing were performed using standard microbiological methods. The BACTEC blood culture analyser detects bacterial growth in the blood culture vials by detecting  $\text{CO}_2$  produced during cell growth, which is translated into reflectometric (fluorescence) units by the automated fluorescence detector [26]. The computer generates growth curves based on plots of fluorescence units (FU) recorded every 10 min versus time. By analysing the generated growth curves, we have learned that, at approximately 50 to 60 min prior to the detection time, the FU start to increase (the log phase) after a period of unchanging values (the lag phase). The shape of the growth curve from the end of the lag phase to the time to detection (TTD) satisfies the following differential equation:

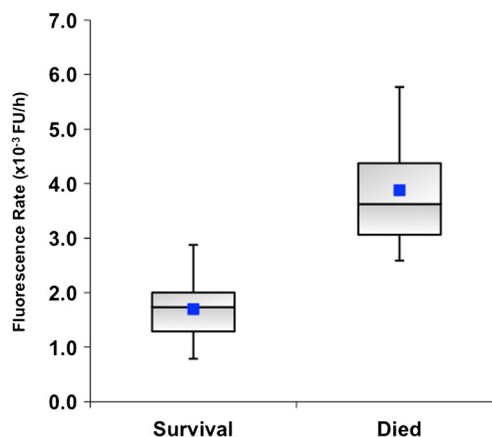
$$\text{Fluorescence rate (FR)} = \Delta F / \Delta T$$

where  $\Delta F$  is the difference in the fluorescence readings between that at the end of the lag phase ( $F_0$ ) and that at the TTD ( $F_x$ ), and  $\Delta T$  is the difference in time between that at the end of the lag phase ( $t_0$ ) and that at the TTD ( $t_x$ ). The above equation was calculated according to the following equation:

$$\Delta F / \Delta T = K \cdot F_0$$

where  $K$  is the rate constant. The rate constant was calculated for each isolate according to the following equation:

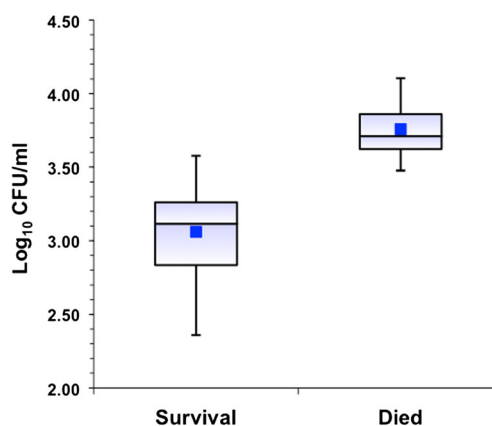
$$F_x = F_0 e^{Kt}$$



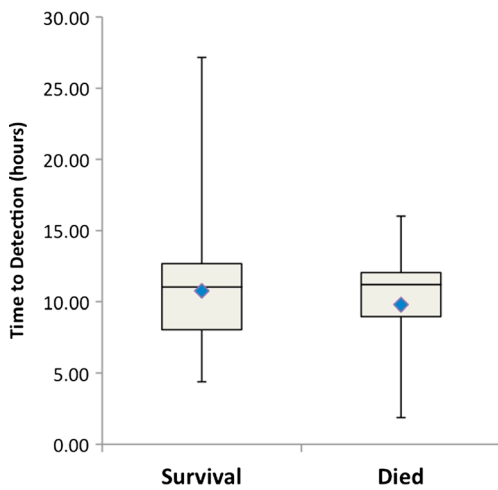
**Fig. 2** FR predicts mortality: comparison between survival and non-survival groups of FR, calculated for the individual Lytic/10 anaerobic blood culture vial for each patient

### Measuring bacterial load

Ten-fold dilutions were performed using the NCTC 12977 strain of *S. pneumoniae*. Known volumes were inoculated into BACTEC Lytic/10 blood culture vials and placed into the blood culture analyser. The fluorescence rate (FR) was calculated for each dilution and plotted against bacterial concentration ( $\log_{10}$  CFU). The logarithmic regression standard curve (CFU vs. FR) for *S. pneumoniae* strain NCTC 12977 (Fig. 1) was generated by plotting the mean of at least four samples of each ten-fold dilution ( $\log_{10}$  CFU/ml) against the corresponding FR. The generated equation of the logarithmic regression curve  $y = 0.9366 \ln(\times 10^{-3} \text{ FU/h}) + 2.5869$  was used to estimate the bacterial load in all blood culture samples. The bacterial load for each of the clinical isolates was measured by exploiting the FR to the corresponding bacterial concentration.



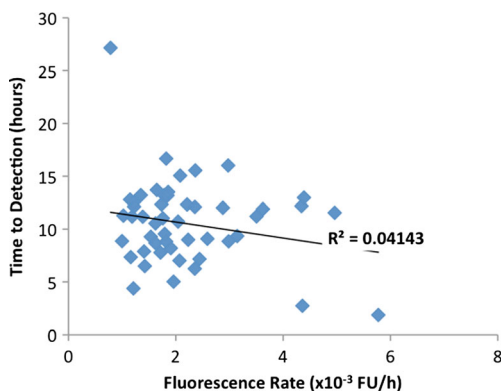
**Fig. 3** Bacterial load predicts mortality: comparison between survival and non-survival groups of bacterial load, calculated using the standard curve generated for *S. pneumoniae* strain NCTC 12977 in Fig. 1



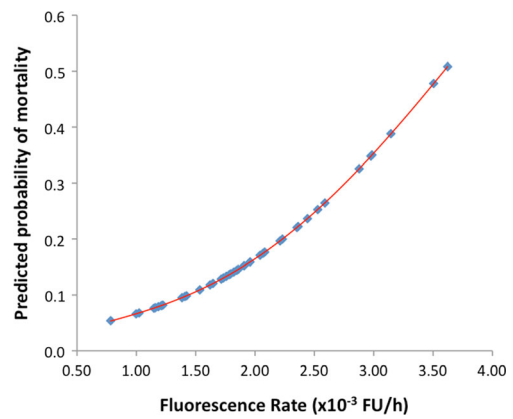
**Fig. 4** Time to detection (TTD) does not predict mortality: comparison between survival and non-survival groups of TTD (h) calculated for the individual Lytic/10 anaerobic blood culture vial for each patient

**Statistical analysis**

Categorical data are presented as frequency and percentages, while continuous data are presented as mean and standard deviation or median and interquartile range, depending on whether the variable conforms to a near normal distribution or not, respectively. Each variable is stratified by mortality and a test is performed comparing the difference between the two categories. The Chi-squared test is used for categorical data, and either the *t*-test or Mann–Whitney *U*-test is performed for continuous data, also dependent on whether they conform to a near normal distribution or not. Univariable logistic regression models were built looking at the association between each predictor variable and mortality. Corresponding multivariable logistic regression models were constructed after age and gender adjustments. Values of *p*<0.05 are considered to be statistically significant. Statistical analysis was performed using STATA 12.1 statistical analysis software (StataCorp, College Station, TX).



**Fig. 5** FR for individual Lytic/10 anaerobic blood culture vials plotted against TTD recorded by the BACTEC analyser for each sample. The FR does not correlate with the TTD



**Fig. 6** Logistic regression analysis for the predicted probability of mortality as a function of FR of *S. pneumoniae* growth in anaerobic Lytic/10 blood culture vials

**Results**

**Clinical characteristics**

Fifty patients were included in the study, having a mean age of 70.6 years (49.6–86.3). Forty patients survived and ten patients died. We identified no statistically significant differences in the demographic or clinical features between the cohort which survived and the cohort which died (Table 1).

The presence of AKI was significantly more common in the patients who did not survive the pneumonia (*p*<0.001; Table 1). The presence of rapid radiological progression and mean PSI score differed significantly between the group that survived and the group that did not (*p*=0.002; Table 1). Despite reaching statistical significance, the crude odds ratio for the mean PSI score between the groups was only 1.03 (*p*=0.007).

**Fluorescence rate of bacterial growth predicts mortality**

The mean FR for the non-survival group ( $3.62 \times 10^{-3}$  FU/h) was significantly higher (*p*<0.001) than that of the survival group ( $1.73 \times 10^{-3}$  FU/h; Table 1 and Fig. 2). For the estimated bacterial load in the patients’ blood samples at the time of

**Table 2** Multivariable logistic regression for clinical history components of the pneumonia severity index (PSI) score and mortality

Variable	Mortality odds ratio	95 % CI	<i>p</i> -Value
Age	1.07	1.00–1.14	0.043
Male	6.11	0.77–48.37	0.087
Neoplastic disease	0.49	0.04–5.77	0.575
Liver disease	12.52	0.66–236.44	0.092
Congestive heart failure	0.36	0.01–10.41	0.551
Cerebrovascular disease	1.72	0.21–13.78	0.609
Renal disease	1	–	–

**Table 3** Univariable logistic regression for clinical history components of the PSI score and fluorescence rate (FR)

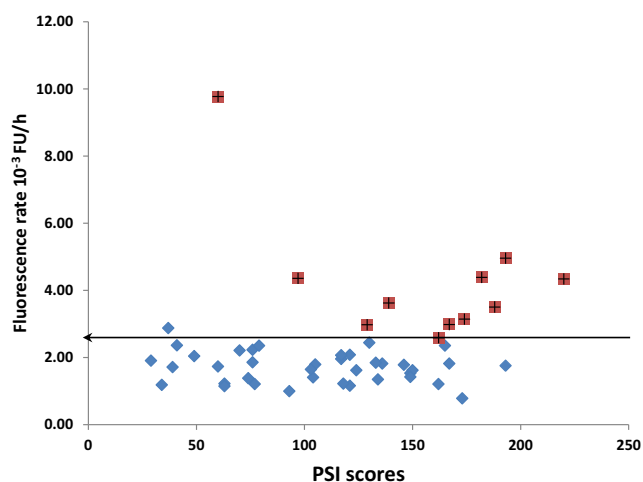
Variable	FR crude coefficient	95 % CI	p-Value
Age	-0.004	(-0.02–0.016)	0.713
Male	0.37	(-0.48–1.22)	0.387
Neoplastic disease	-0.44	(-1.58–0.68)	0.429
Liver disease	2.01	(0.59–3.43)	0.007
Congestive heart failure	0.64	(-1.48–2.76)	0.546
Cerebrovascular disease	0.003	(-1.13–1.14)	0.996
Renal disease	-0.23	(-1.62–1.15)	0.737

taking the blood culture, the mean for the non-survival group ( $3.76 \pm 0.06 \log_{10}$  CFU/ml) was significantly higher ( $p < 0.001$ ) than that of the survival group ( $3.06 \pm 0.04 \log_{10}$  CFU/ml; Fig. 3).

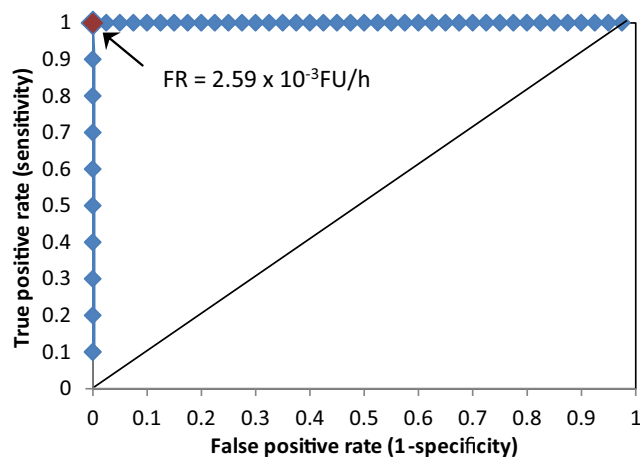
The TTD did not predict mortality. The mean TTD for the survival group [ $10.04 \pm 0.59$  h; 95 % confidence interval (CI), 8.89 to 11.19 h] was not significantly different ( $p = 0.94$ ) from that of the non-survival group ( $10.14 \pm 1.52$  h; 95 % CI, 7.15 to 13.13 h; Fig. 4). There was no correlation between the FR and the TTD (Fig. 5).

### Fluorescence rate quantifies mortality risk

The relationship between the FR and the probability of mortality is described in Fig. 6. Logistic regression analysis showed a small but statistically significant association of age with mortality (Table 2). However, the FR is not independently associated with key clinical components of the PSI score (Table 3). The discriminative ability of the FR to differentiate between survivals and non-survivals was evaluated with area under the receiver operating characteristic (AUROC) curve



**Fig. 7** Pneumonia severity index (PSI) scores for individual patients plotted against individual FR. The FR at a proposed cut-off value ( $2.59 \times 10^{-3}$  FU/h) predicts mortality more accurately than the PSI scores: *diamond* survival and *crosses* non-survival



**Fig. 8** Receiver operating characteristic (ROC) curve showing the accuracy of the FR as a predictable marker for risk of mortality

analysis. The FR was an excellent marker for the determination of the risk of mortality (AUROC 0.98). We determined that the optimal cut-off for high risk of mortality is  $2.59 \times 10^{-3}$  FU/h (Figs. 7 and 8), with a sensitivity of 91 % (CI, 62 to 98 %) and a specificity of 97 % (CI, 86 to 99 %).

### Conclusions

Our small retrospective cohort study describes a novel application of the BACTEC blood culture analyser to predict mortality in patients with invasive pneumococcal pneumonia. We found that the fluorescence rate (FR), calculated from routine blood culture analysis, correlated closely with the probability of mortality in this patient group. Our data suggest that, across different strains of *Streptococcus pneumoniae*, the FR is an accurate surrogate for bacterial load. These findings concord with existing data that describe the strong relationship between the bacterial load of invasive pneumococcal infections and clinical outcome [20, 21].

Our results suggest that calculation of the FR as part of routine blood culture work-up for suspected pneumococcal sepsis may help stratify the severity of illness, thus guiding appropriate escalation of clinical care. It is well recognised that current clinical and microbiological tools fail to achieve this reliably in the setting of acute medical admissions [13, 16–19]. Our extrapolated analysis of existing culture techniques is cheap, widely available and can be integrated into the automated process of the BACTEC blood culture analyser. The software required to optimise the BACTEC system adds negligible cost and an FR report can be generated instantaneously. Manual calculation of the FR, without additional software, takes less than 5 min. Our innovative approach may have a role in other invasive pneumococcal infections, such as meningitis, but also in sepsis caused by different organisms, where objective and early assessment of disease

severity could improve clinical outcome. In time, a specific FR threshold for different organisms may provide a single value that accurately prognosticates sepsis outcomes.

Our study is necessarily limited by the small sample size and its retrospective design. Bacterial load based on blood culture inoculation, and, consequently, the FR, is directly affected by preceding antibiotic administration. As far as possible in our study, we have excluded patients receiving antibiotic therapy prior to blood culture collection. However, owing to individual variation in the documentation of clinical notes, we cannot definitively report that all patients did not receive oral antibiotic preparations prior to hospital admission. In contrast, the sensitivity of DNA assays does not significantly change over days after antibiotic administration [23]. Six patients out of the ten patients who died due to invasive pneumococcal pneumonia were intentionally not escalated to intensive care or to intubation as part of the clinical plan. It may be the case that death may be preventable in patients where escalation is clinically appropriate. However, we acknowledge that this may have a compromising effect on the correlation of the FR to mortality. Alternatively, there may be unrecognised clinical or non-clinical parameters, not identified by our linear regression analyses, which predispose to the high mortality in this specific group of patients. However, the survival and non-survival groups demonstrated no statistically significant variation in the demographic or clinical profiles. With the two groups matched in this way, it suggests that the FR remains a prognostic tool of high utility. Similarly, while delays in presentation to hospital might also predict poor prognosis, owing to the untreated complications of sepsis, we could not accurately capture these data from the clinical notes audit. However, at presentation, the bacterial load and FR will also increase with prolonged and uninhibited bacterial growth. Thus, these analyses will still likely provide a clinically useful snapshot of disease severity at the time of blood culture sampling.

The ideal diagnostic tool in the setting of any presentation of sepsis provides organism identification and measure of disease severity as early as possible during admission when mortality is highest [9]. One limitation of the FR is that it provides discriminating results only as quickly as blood culture, which is slower than potential future molecular tests. We found that the time to detection (TTD) did not correlate with clinical outcomes in our cohort. In data not shown, we found that the TTD varied significantly according to isolate serotype, whereas the FR did not. The FR does not appear to be strain-dependent and provides a consistent measure of bacterial load.

In conclusion, bacterial load is known to predict clinical outcome in invasive pneumococcal pneumonia. Our innovation of FR calculation using routine blood culture techniques correlates with bacterial load and predicts mortality in a small retrospective cohort study. The implications for this simple and universal tool may extend beyond stratifying severity in

patients with invasive pneumococcal disease (IPD) to support clinical decisions for all causes of sepsis with detectable bacteraemia.

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**Conflict of interest** The authors declare no potential conflicts of interest.

**Compliance with ethical standards** This study is compliant with ethical standards and did not require informed consent.

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