Supplementary Data

Bacteraemia and "Antibiotic Resistant Pathogens" Bacteraemia in Community Acquired Pneumonia: Risk and Prognosis

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MATERIALS AND METHODS

Study design and patients

The exclusion criteria were: a) severe immunosuppression (AIDS, chemotherapy, immunosuppressive drugs (e.g., oral corticosteroid ≥10mg prednisone or equivalent per day for at least two weeks)), b) active tuberculosis, c) cases with a confirmed alternate diagnosis. Non-hospitalized patients were re-examined after 1-7 days in the outpatient clinic.

Data collection

Current smoking (>10 pack-years), alcohol habits (ingestion of an estimated amount of >80g alcohol per day for at least one year before presentation), co-morbidities (chronic respiratory disease, including chronic obstructive pulmonary disease, asthma and bronchiectasis among others, diabetes mellitus, chronic cardiovascular disease, neurological disease, chronic renal disease, chronic liver disease), nursing-home, clinical symptoms and features (fever, cough, pleuritic pleuritic pain, dyspnoea, mental confusion, and aspiration), clinical signs (blood pressure, body temperature, respiratory rate, and heart rate), chest radiograph findings (number of lobes affected, pleural effusion, and atelectasis), laboratory parameters (haemoglobin level, white-blood-cell count, platelet count, serum creatinine level, C-reactive protein level, and other biochemical parameters), pulmonary complications (empyema, acute respiratory distress syndrome criteria, pleural effusion, surgical pleural draining), clinical events (cardiac arrhythmias, septic shock, acute renal failure).

Criteria for etiological diagnosis

The aetiology was considered definite if one of the following criteria was met: 1) blood culture positive (in the absence of an apparent extra-pulmonary focus); 2) positive bacterial culture of pleural fluid or transthoracic needle aspiration samples; 3) elevated serum levels of IgM against *Chlamydophila pneumoniae* (≥1:64), *Coxiella burnetii* (≥1:80) and *Mycoplasma pneumoniae* (any positive titre); 4) seroconversion (i.e., a fourfold increase in IgG titres) for *Chlamydophila pneumoniae* and *Legionella pneumophila* >1:128, *C. burnetii* >1:80 and respiratory viruses (influenza viruses A and B, parainfluenza viruses 1-3, respiratory syncytial virus, adenovirus); 5) positive urinary

antigen for *Legionella pneumophila* (Binax Now *Legionella pneumophila* urinary Antigen Test; Trinity Biotech, Bray, Ireland); 6) positive urinary antigen for *Streptococcus pneumoniae* (Binax Now *Streptococcus pneumoniae* urinary Antigen Test; Emergo Europe, The Hague, The Netherlands); 7) bacterial growth in cultures of TBAS ($\geq 10^5$cfu/ml), in protected specimen brush ($\geq 10^3$cfu/ml), or BAL ($\geq 10^4$cfu/ml); 8) detection of antigens by immunofluorescence assay (IFA) plus virus isolation or detection by reverse transcriptase polymerase chain reaction (RT-PCR) testing for respiratory virus (Influenza viruses A and B, parainfluenza *virus* 1 to 3, respiratory syncytial virus, rhinovirus, adenovirus).

The aetiology of pneumonia was classified as presumptive when a predominant microorganism was isolated from a purulent sample (leukocytes >25 per high power microscopic field and <10 epithelial cells per high power microscopic field) and the findings of Gram staining were compatible. For the purpose of this study presumptive and definitive diagnostic were analyzed together.

Statistical analysis

Univariate and multivariate logistic regression analyses were performed to identify variables predictive of patients with bacteraemic CAP and patients difficult to treat bacteraemia, respectively (dependent variables). The variables analyzed univariately were influenza vaccination, pneumococcal vaccination, prior antibiotic treatment, chronic pulmonary disease, diabetes mellitus, neurological disease, pleuritic pain, nursing-home, PSI risk class (I-III vs. IV-V), CURB-65 (1-2 vs. 3-5), C-reactive protein (<21.6 vs. ≥21.6 mg/dL for the analyses of patients with bacteraemic CAP and <22.2 vs. ≥22.2 mg/dL for the analyses of patients difficult to treat bacteraemia), white-blood-cell count (<10 vs. ≥10 x 10^9 cell/L), intensive care unit (ICU) admission and mechanical ventilation. Also univariate and multivariate logistic regression analyses were performed to predict 30-day hospital mortality (dependent variable); we also assessed a subgroup analysis for patients with bacteraemia. The variables analyzed univariately were those mentioned above plus cough, sputum, dyspnea, saturation (<92 vs. ≥92 %), PaO_2/FiO_2 (<250 vs. ≥250), appropriate empiric treatment and bacteraemia. Receiver operating characteristic (ROC) curves were constructed to determine the best cut-off points for C-reactive protein.

RESULTS

Patients who had blood cultures done were more current smokers and current alcohol consumers, less frequently received prior antibiotic treatment, presented more fever and pleuritic pain and had less frequent neurological disease. Also they were less nursing-home, presented elevated C-reactive protein, creatinine and white-blood-cell count, need more ICU admission, have longer length of stay and presented lower rate of 30-day mortality.

Table S1. Clinical and epidemiological characteristics of patients receiving and not receiving blood culture

Variable	Non-done blood culture (n = 827)	Done blood culture (n = 2892)	p-value
Demographic			
Age (years)	68.0 (19.6)	64.0 (18.4)	**<0.001**
Age ≥65 years	529 (64.0)	1691(58.5)	**0.005**
Men	472 (57.1)	1803 (62.3)	**0.006**
Smoking			**0.006**
No smoker	395 (48.4)	1220 (42.4)	**0.002**
Current smoker	175 (21.4)	733 (25.5)	**0.019**
Ex-smoker	246 (30.1)	925 (32.1)	0..28
Alcohol			**0.024**
No alcohol consumer	688 (84.9)	2317 (80.8)	**0.007**
Current alcohol consumer	978 (12.0)	425 (14.8)	**0.040**
Ex-alcohol consumer	25 (3.1)	125 (4.4)	0.10
Previous antibiotic	204 (26.3)	595 (21.4)	**0.004**
Influenza vaccine	274 (41.1)	1041 (43.3)	0.31
Pneumococcal vaccine	88 (13.3)	389 (16.2)	0.060
Inhaled corticosteroid	144 (17.7)	512 (17.9)	0.88
Systemic corticosteroid	16 (2.1)	39 (1.5)	0.25
Symptoms			
Fever	557 (71.6)	2372 (85.8)	**0.001**
Pleuritic pain	301 (37.4)	1205 (42.5)	**0.010**
Comorbidities*	185 (62.3)	1643 (63.3)	0.73

Variable	Non-done blood culture (n = 827)	Done blood culture (n = 2892)	p-value
Chronic respiratory disease	331 (40.0)	1076 (37.2)	0.14
Chronic cardiovascular disease	159 (19.3)	496 (17.3)	0.17
Diabetes mellitus	124 (15.7)	469 (16.8)	0.44
Neurological disease	169 (20.7)	490 (17.1)	**0.017**
Chronic renal disease	49 (6.0)	181 (6.3)	0.72
Chronic liver disease	39 (4.8)	125 (4.3)	0.62
Nursing-home	32 (3.9)	61 (2.1)	**0.004**
Laboratory parameters			
Creatinine (mg/dL)	1.0 (0.8-1.4)	1.1 (0.9-1.4)	**0.008**
C-reactive protein (mg/dL)	11.7 (5.8-21.5)	18.7 (10.0-27.8)	**<0.001**
White-blood-cell count (10^9/L)	11.8 (8.6-16.1)	12.9 (9.1-17.7)	**<0.001**
Sat 0_2 (%)	93.5 (89.9-96.0)	93.6 (90.6-95.8)	0.70
PaO_2/FiO_2	285.7 (242.7-333.3)	285.7 (242.9-333.3)	0.68
CURB-65 risk class 3-5	120 (15.9)	512 (18.5)	0.10
PSI risk class IV-V	419 (50.7)	1427 (49.3)	0.50
ICU admission	76 (9.2)	538 (18.6)	**<0.001**
Mechanical ventilation[$]			**0.002**
Not ventilated	784 (94.8)	2645 (91.5)	**0.001**
Non-invasive	26 (3.1)	110 (3.8)	0.40
Invasive	17 (2.1)	137 (4.7)	**0.001**
Length of hospital stay (days)	6.0 (1.5-9.0)	7.0 (4.0-10.0)	**<0.001**
30-day mortality	78 (9.4)	187 (6.5)	**0.003**

Data are number of patients (%), mean (SD) or median (1st quartile-3rd quartile). Percentages calculated on non-missing data. CURB-65=consciousness, urea, respiratory rate, blood pressure, 65 years old. ICU=intensive care unit. PSI=pneumonia severity index. * Patients could have more than one comorbidity. [$] Patients who received initially non-invasive ventilation but needed subsequently intubation were included in the invasive mechanical ventilation group.

Table S2. Clinical and epidemiological characteristics of bacteraemic and non-bacteraemic CAP patients

Variable	Bacteraemic CAP (n = 297)	Non-bacteraemic CAP (n = 2595)	p-value
Demographic			
Age (years)	63.1 (19.0)	64.8 (18.8)	0.11
Age ≥65 years	156 (52.5)	1535 (59.2)	**0.028**
Men	167 (56.2)	1636 (63.0)	**0.022**
Smoking			0.073
No smoker	133 (45.4)	1087 (42.1)	0.27
Current smoker	83 (28.3)	650 (25.1)	0.24
Ex-smoker	77 (26.3)	848 (32.8)	**0.023**
Alcohol			0.42
No alcohol consumer	229 (78.2)	2088 (81.1)	0.22
Current alcohol consumer	48 (16.4)	377 (14.6)	0.43
Ex-alcohol consumer	16 (5.5)	109 (4.2)	0.33
Previous antibiotic	35 (12.5)	560 (22.4)	**<0.001**
Influenza vaccine	83 (37.4)	958 (43.9)	0.062
Pneumococcal vaccine	22 (10.0)	367 (16.9)	**0.008**
Inhaled corticosteroid	50 (17.2)	462 (18.0)	0.74
Systemic corticosteroid	3 (1.1)	36 (1.5)	0.61
Symptoms			
Cough	238 (82.6)	2041 (79.7)	0.24
Fever	255 (89.8)	2117 (85.3)	**0.041**
Purulent sputum	173 (61.6)	1460 (58.1)	0.26
Pleuritic pain	171 (60.0)	1034 (40.5)	**<0.001**
Dyspnoea	200 (69.4)	1663 (65.2)	0.15
Altered mental status	66 (22.3)	450 (17.5)	**0.040**
Comorbidities*	185 (62.3)	1643 (63.3)	0.73
Chronic respiratory disease	108 (36.4)	968 (37.3)	0.75
Chronic obstructive pulmonary disease	36 (12.1)	402 (15.5)	0.13
Chronic cardiovascular disease	37 (12.5)	459 (18.8)	**0.024**
Diabetes mellitus	52 (18.2)	417 (16.6)	0.51
Neurological disease	52 (17.7)	438 (17.0)	0.78

Variable	Bacteraemic CAP (n = 297)	Non-bacteraemic CAP (n = 2595)	p-value
Chronic renal disease	16 (5.4)	165 (6.4)	0.52
Chronic liver disease	18 (6.1)	107 (4.1)	0.12
Nursing-home	56 (2.2)	5 (1.7)	0.59
Laboratory parameters			
Creatinine (mg/dL)	1.2 (0.9-1.6)	1.0 (0.9-1.4)	**<0.001**
Creatinine ≥1.5 mg/dL	97 (32.9)	518 (20.2)	**<0.001**
C-reactive protein (mg/dL)	25.5 (15.5-33.9)	18.0 (9.6-27.0)	**<0.001**
C-reactive protein ≥21.6 mg/dL	152 (63.1)	834 (39.2)	**<0.001**
White-blood-cell count (10^9/L)	15.9 (9.8-20.4)	12.6 (9.0-17.3)	**<0.001**
White-blood-cell count ≥10 x 10^9/L	219 (74.7)	1733 (67.8)	**0.015**
Sat 0_2 (%)	93.0 (89.5-95.0)	93.8 (90.9-95.9)	**0.003**
Sat 0_2 <92 %	75 (39.5)	492 (32.0)	**0.039**
PaO_2/FiO_2	271.4 (233.3-304.8)	288.3 (247.6-333.3)	**<0.001**
PaO_2/FiO_2 <250	88 (38.8)	495 (26.9)	**<0.001**
CURB-65 risk class 3-5	76 (25.9)	436 (17.6)	**0.001**
PSI risk class IV-V	166 (55.9)	1261 (48.6)	**0.017**
ICU admission	93 (31.3)	445 (17.1)	**<0.001**
Mechanical ventilation[$]			**0.001**
Not ventilated	255 (85.9)	2390 (92.1)	**<0.001**
Non-invasive	18 (6.1)	92 (3.5)	**0.032**
Invasive	24 (8.1)	113 (4.4)	**0.004**
Length of hospital stay (days)	9.0 (6.0-13.0)	6.0 (4.0-10.0)	**<0.001**
30-day mortality	33 (11.1)	154 (5.9)	**0.001**
Appropriate empiric treatment	282 (94.9)	2446 (95.2)	0.86

Data are number of patients (%), mean (SD) or median (1st quartile-3rd quartile). Percentages calculated on non-missing data. CURB-65=consciousness, urea, respiratory rate, blood pressure, 65 years old. ICU=intensive care unit. PSI=pneumonia severity index. * Patients could have more than one comorbidity. [$] Patients who received initially non-invasive ventilation but needed subsequently intubation were included in the invasive mechanical ventilation group.

Microbial aetiology and susceptibility

Two hundred forty six isolates of p*neumococcus* were tested against penicillin, 221 (90%) were susceptible (MIC ≤0.06 μg/mL), and 25 (10%) were resistant (MIC ≥2

µg/mL); two hundred forty one isolates were tested against erythromycin, 206 (85%) isolates were susceptible and 35 (15%) were resistant; fifty one isolates were tested for cefotaxime, 49 (96%) were susceptible and 2 (4%) were resistant. Two isolates of *Staphylococcus aureus* (12%) were methicillin susceptible (MSSA) and 14 (88%) were methicillin resistant (MRSA). The 1 isolate of *Pseudomonas aeruginosa* was susceptible to anti-Pseudomonal antibiotics. 6 isolates of *Escherichia coli*, 2 isolates of *Klebsiella pneumoniae*, 1 isolate of *Enterobacter species* and the 1 isolate of *Proteus species* were beta-lactamase producers.

Table S3. Bacteriological findings of 1292 patients with microbial aetiology

Isolate	Total n=1292 n %	Blood Culture	Urinary antigen	Sputum	BAS/BAL	Pleural fluid
Streptococcus pneumoniae	567 (43.9)	249	200	60	40	20
*Streptococcus pneumoniae multiresistant**		5	-	2	1	1
Staphylococcus aureus	23 (1.8)	16	-	10	8	-
*MRSA**		14	-	2	6	-
MSSA		2	-	1	2	-
Escherichia coli	12 (0.9)	9	-	2	-	1
*Escherichia coli BL producers**		6	-	1		-
Haemophilus influenzae	37 (2.9)	7	-	26	5	-
Enterobacter species	8 (0.6)	4	-		4	-
*Enterobacter species BL producers**	-	1	-	-	1	-
Klebsiella pneumoniae	5 (0.4)	4	-	1	1	-
*Klebsiella pneumoniae BL producers**	-	2	-	1	-	-

Isolate	Total n=1292 n %	Blood Culture	Urinary antigen	Sputum	BAS/BAL	Pleural fluid
Acinetobacter baumannii	2 (0.2)	2	-	-	-	-
Proteus species	2 (0.2)	2	-	-	-	-
*Proteus species BL producers**	1	1	-	-	-	-
Moraxella catarrhalis	5 (0.4)	1	-	4	-	-
Peptostreptococcus sp	1 (0.1)	1	-	-	-	1
*Pseudomonas aeruginosa**	24 (1.9)	1	-	16	8	1
Streptococcus pyogenes	4 (0.3)	1	-	-	1	2
Respiratory viruses	181 (14.0)	-	-	-	-	-
Polymicrobial aaetiology	174 (13.5)	-	-	-	-	-
Atypical	99 (7.7)	-	-	-	-	-
Legionella pneumophila	97 (7.5)	-	97	-	-	-
Other	51 (4.0)	-	-	-	-	-

Data are number of patients. *MRSA=methicillin resistant Staphylococcus aureus*. *MSSA=methicillin sensitive Staphylococcus aureus*

Empiric antibiotic therapy

Patients with bacteraemic CAP received the following antibiotics: beta-lactam plus macrolide (n=116, 39%), beta-lactam plus fluoroquinolone (n=93, 31%), fluoroquinolone monotherapy (n=39, 13%), beta-lactam monotherapy (n=13, 4%), fluoroquinolone plus macrolide (n=5, 2%), other combination (n=31, 10%).

When compared to non-bacteraemic CAP, patients with bacteraemia received the combination of a beta-lactam plus fluoroquinolone more often (31% vs. 23%, p=0.001) on admission, and fluoroquinolone monotherapy less often (13% vs. 26%, p<0.001).

Table 4. - Bacteraemic cases that received initial inappropriate treatment (n=15 cases)

***Escherichia coli*:** 4 cases

1. Case 1: Empiric treatment Levofloxacin /*E. coli* resistance to ciprofloxacin
2. Case 2: Empiric treatment Levofloxacin + cefepime/ *E. coli* resistance to ciprofloxacin, intermediate resistance to cefotaxime and cefuroxime
3. Case 3: Empiric treatment Levofloxacin + ceftriaxone/ *E. coli* resistance to ciprofloxacin, intermediate resistance to cefotaxime
4. Caso 4: Empiric treatment Levofloxacin + Tazocel/ *E. coli* resistance to ciprofloxacin and Tazocel

***Staphylococcus aureus*:** 4 cases

1. Case 1: Empiric treatment Ceftriaxone / S.auresus (MRSA)
2. Case 2: Empiric treatment Levofloxacin + Clindamycin/ S. aureus intermediate resistance to Clindamycin + Levofloxacin
3. Case 3: Empiric treatment Levofloxacin + azythromycin / S. aureus (MRSA) intermediate resistance to levofloxacin
4. Case 4: Empiric treatment Ceftriaxone + Levofloxacin / S. aureus (MRSA) resistance to levofloxacin + penicillin

***Haemophilus influenzae*:** 2 cases

1. Case 1: Empiric treatment Levofloxacin /*H. influenzae* beta-lactamase producer intermediate resistance to ciprofloxacin
2. Case 2: Empiric treatment Moxifloxacino/ *H. influenzae* beta-lactamase producer, intermediate resistance to ciprofloxacin

Klebsiella pneumoniae: 2 cases

1. Case 1: Empiric treatment Levofloxacin/ *K. pneumoniae* intermediate resistance to ciprofloxacin + cefazoline
2. Case 2: Empiric treatment Levofloxacin / *K. pneumoniae* intermediate resistance to ciprofloxacin + cefazoline

***Microbiological annotation of laboratory: The MIC of ciprofloxacin suggested a DNA gyrase mutation, avoid the use of fluoroquinolones**

Streptococcus pneumoniae*:* 1 case
Case 1: Empiric treatment levofloxacin + azythromycin / *S. pneumoniae* resistant to erythromycin, intermediate resistance to levofloxacin

Proteus sp: Case 1: Empiric treatment amoxicillin÷clavulanic acid. / *Proteus sp*. resistant to amoxicillin÷clavulanic acid, Ampicillin, Ciprofloxacin, Gentamicin

Acinetobacter sp*:* Case1: Empiric treatment Ceftriaxone + azythromycin / multiresistant *Acinetobacter sp.*

Table S5. Significant univariate and multivariate logistic regression analyses for the prediction of 30-day hospital mortality in patients with bacteraemic CAP

Variable	Univariate			Multivariate*		
	OR	95% CI	p-value	OR	95% CI	p-value
Previous antibiotic	2.68	1.05-6.87	0.040	-	-	-
Neurological disease	3.70	1.70-8.05	0.001	-	-	-
Cough	0.42	0.18-0.98	0.045	0.33	0.12-0.93	0.035
Dyspnoea	3.28	1.11-9.67	0.032	4.64	1.24-17.42	0.023
Pleuritic pain	0.50	0.22-1.11	0.088	-	-	-
White-blood-cell count <10 x 10^9/L	1.93	0.89-4.16	0.095	-	-	-
PaO_2/FIO_2 <250	2.91	1.34-6.63	0.007	-	-	-
PSI risk class IV-V	31.04	4.18-230.52	0.001	26.18	3.34-205.01	0.002
CURB-65 risk class 3-5	5.60	2.63-11.96	<0.001	-	-	-
ICU admission	3.48	1.66-7.31	0.001	2.47	1.05-5.79	0.038
Mechanical ventilation[$]			<0.001	-	-	-
Not ventilated	1	-	-	-	-	-
Non-invasive	0.82	0.10-6.57	0.85	-	-	-
Invasive	23.33	8.92-61.05	<0.001	-	-	-
Length of hospital stay (+7 days)[#]	1.13	0.98-1.31	0.090	-	-	-
Appropriate empiric treatment	0.22	0.07-0.69	0.009	0.19	0.05-0.76	0.019
ARP bacteraemia	2.96	1.15-7.60	0.024	-	-	-

CI=confidence interval. CURB-65=consciousness, urea, respiratory rate, blood pressure, 65 years old. ICU=intensive care unit. OR=odds ratio. PSI=pneumonia severity index. ARP= Antibiotic Resistant Pathogens * Hosmer-Lemeshow goodness-of-fit test, p=0.98. [$] The p-value corresponds to differences between the three groups (not ventilated, non-invasive or invasive). [#] Length of hospital stay was treated as a continuous variable and +7 days indicates the increase by seven days.

Supplementary Figures

Bacteraemia and "Antibiotic Resistant Pathogens" Bacteraemia in Community Acquired Pneumonia: Risk and Prognosis

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Figures Legend:

Figure S1: **Bacteraemia by site of care**

Figure S2: **Bacteraemia by Pneumonia Severity Index score**

Figure S3: **Bacteraemia by CURB-65 score**

Figure S4: **ROC analysis of significant variables derived from the logistic regression model in their capacity to predict Bacteraemia**

Figure S5: **ROC analysis of significant variables derived from the logistic regression model in their capacity to predict 30-day hospital mortality**

Figure S6: **ROC analysis of significant variables derived from the logistic regression model in their capacity to predict 30-day hospital mortality in patients with bacteraemic CAP**