ICU-Acquired Pneumonia With or Without Etiologic Diagnosis: A Comparison of Outcomes*

Valeria Giunta, MD[1,2,3]; Miquel Ferrer, MD, PhD[1,2]; Mariano Esperatti, MD[1,2];
Otavio T. Ranzani, MD[1,2,4]; Lina Maria Saucedo, MD[1]; Gianluigi Li Bassi, MD, PhD[1,2];
Francesco Blasi, MD, PhD[3]; Antoni Torres, MD, PhD[1,2]

Objectives: The impact of ICU-acquired pneumonia without etiologic diagnosis on patients' outcomes is largely unknown. We compared the clinical characteristics, inflammatory response, and outcomes between patients with and without microbiologically confirmed ICU-acquired pneumonia.
Design: Prospective observational study.
Setting: ICUs of a university teaching hospital.
Patients: We prospectively collected 270 consecutive patients with ICU-acquired pneumonia. Patients were clustered according to positive or negative microbiologic results.
Interventions: None.
Measurements and Main Results: We compared the characteristics and outcomes between both groups. Negative microbiology was found in 82 patients (30%). Both groups had similar baseline severity scores. Patients with negative microbiology presented more frequently chronic renal failure (15 [18%] vs 11 [6%]; $p = 0.003$), chronic heart disorders (35 [43%] vs 55 [29%];

$p = 0.044$), less frequently previous intubation (44 [54%] vs 135 [72%]; $p = 0.006$), more severe hypoxemia (Pao_2/Fio_2: 165 ± 73 mm Hg vs 199 ± 79 mm Hg; $p = 0.001$), and shorter ICU stay before the onset of pneumonia (5 ± 5 days vs 7 ± 9 days; $p = 0.001$) compared with patients with positive microbiology. The systemic inflammatory response was similar between both groups. Negative microbiology resulted in less changes of empiric treatment (33 [40%] vs 112 [60%]; $p = 0.005$) and shorter total duration of antimicrobials (13 ± 6 days vs 17 ± 12 days; $p = 0.006$) than positive microbiology. Following adjustment for potential confounders, patients with positive microbiology had higher hospital mortality (adjusted odds ratio 2.96, 95% confidence interval 1.24–7.04, $p = 0.014$) and lower 90-day survival (adjusted hazard ratio 0.50, 95% confidence interval 0.27–0.94, $p = 0.031$), with a nonsignificant lower 28-day survival.
Conclusions: Although the possible influence of previous intubation in mortality of both groups is not completely discarded, negative microbiologic findings in clinically suspected ICU-acquired pneumonia are associated with less frequent previous intubation, shorter duration of antimicrobial treatment, and better survival. Future studies should corroborate the presence of pneumonia in patients with suspected ICU-acquired pneumonia and negative microbiology. (*Crit Care Med* 2013; 41:2133–2143)
Key Words: ICU; lung; microbiology; nosocomial infection; ventilator-associated pneumonia

ICU-acquired pneumonia (ICUAP), which includes ventilator-associated pneumonia (VAP) and nonventilator ICU-acquired pneumonia (NV-ICUAP), is the leading infection in critically ill patients, accounting for prolonged mechanical ventilation and length of stay, and poor outcome (1–4). Although the mortality rate of ICUAP is variable among studies, and the prognostic impact of VAP in terms of attributable mortality has been recently questioned (5), there is general agreement that an early etiologic diagnosis and a timely and appropriate antibiotic treatment may contribute to reduce complications and mortality (6–9).

According to current guidelines, the management strategies for patients with suspected ICUAP should include the

collection of a lower respiratory tract (LRT) sample for culture, start empiric antimicrobial therapy unless there are both a low clinical suspicion for pneumonia and a negative microscopic examination of the LRT sample, and finally adjust antibiotic therapy according to microbiologic results (1).

A difficult target for the etiologic diagnosis of ICUAP is the absence of a gold standard test (1, 10–12). The purpose of diagnostic testing is to define whether a patient has actually pneumonia and to determine the etiologic pathogen when pneumonia is present. Unfortunately, currently available tools cannot always reliably provide this information. Diagnosis is difficult, especially in nonintubated patients (2); hence, the reliability of the bacteriologic information is uncertain and the specificity of the diagnosis undefined (1, 13). Furthermore, the diagnostic accuracy of microbiologic samples is affected by the recent introduction of antibiotic treatment (14). Therefore, in the presence of negative microbiologic samples, pneumonia cannot be definitively ruled out and physicians often treat patients according to clinical judgment independently of microbiologic results.

To our knowledge, there are no studies that have compared populations with clinical suspicion of ICUAP confirmed or not by microbiologic data to guide physicians in clinical practice. The objective of our study was to compare the characteristics and outcomes of patients with clinical suspicion of ICUAP with positive and negative microbiologic samples in a real-life ICU population.

METHODS

Study Population

The study was conducted in six medical and surgical ICUs, overall comprising 45 beds, of an 800-bed university hospital. Data were prospectively collected from January 2007 to April 2011. The investigators made daily rounds in each ICU. Patients older than 18 years, admitted to these ICUs for 48 hours or more, with clinical suspicion of ICUAP were consecutively included into the study and only the first episode was analyzed. Patients with severe immunosuppression (neutropenia after chemotherapy or hematopoietic transplant, drug-induced immunosuppression in solid-organ transplant or cytotoxic therapy, and HIV-infected patients) (15), absence of LRT sample collected, and patients with new antibiotic treatment in the last 72 hours prior to diagnosis and negative microbiology were excluded. The institution's Internal Review Board approved the study (*Comite Etic d'Investigacio Clinica*, registry number 2009/5427), and written informed consent was obtained from patients or their next-of-kin.

Definition of Pneumonia, Microbiologic Processing, and Antimicrobial Treatment

The clinical suspicion of pneumonia was based on either clinical criteria (new or progressive radiological pulmonary infiltrate together with at least two of the following: 1) temperature > 38°C or < 36°C; 2) leukocytosis > 12,000/mm^3 or leucopoenia < 4,000/mm^3; or 3) purulent respiratory secretions) (1, 16, 17) or a simplified Clinical Pulmonary Infectious Score (CPIS) greater than 6 (18).

The microbiologic evaluation included the collection of at least one lower respiratory airways sample: sputum in nonventilated patients, tracheobronchial aspirates (TBAs) in intubated patients, and/or bronchoscopic (19) or blind bronchoalveolar lavage (BAL) (20) if possible, within the first 24 hours of inclusion (21). Only samples of sputum or tracheal aspirates of high quality (i.e., < 10 squamous cells and > 25 leukocytes per optical microscopy field) were processed for culture. The same sampling method was performed on the third day if clinically indicated. Blood cultures and cultures from pleural fluid if puncture was indicated were also taken. Urinary antigens of *Streptococcus pneumoniae* and *Legionella pneumophila* were not systematically collected. Microbiologic confirmation of pneumonia was defined by the presence of at least one potentially pathogenic microorganism (PPM), that is, endogenous microorganisms associated with nosocomial respiratory infection in the host (22), in respiratory samples above predefined thresholds (BAL >10^4, sputum or TBAs >10^5 colony-forming units/mL, respectively, or any threshold if the patient had antibiotic treatment), in pleural fluid, or in blood cultures if an alternative cause of bacteremia was ruled out (23, 24). Microbiologic identification and susceptibility testing were performed by
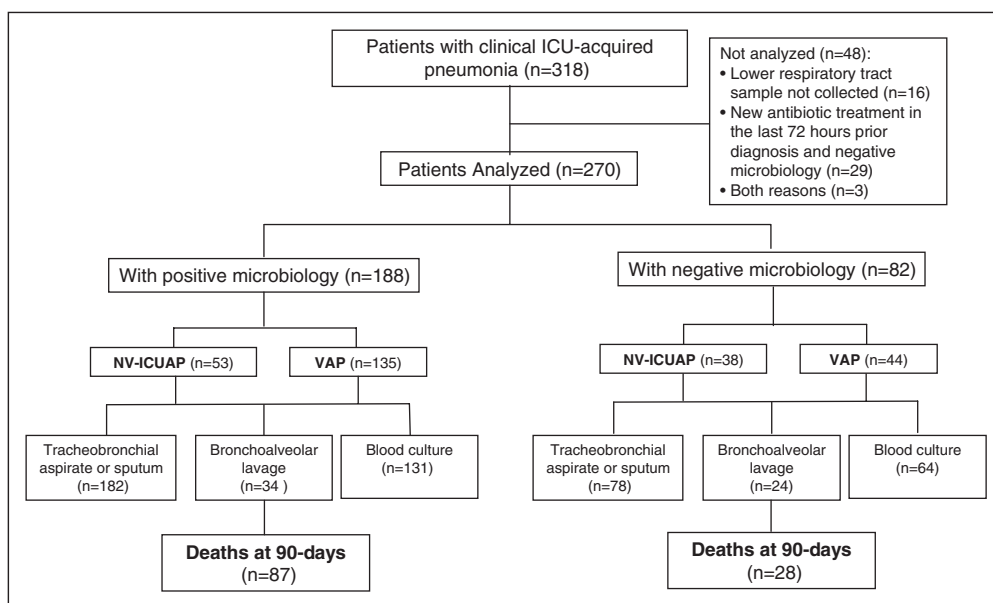
Figure 1. Flow chart of the cohort. NV-ICUAP = nonventilator ICU-acquired pneumonia, VAP = ventilator-associated pneumonia.

TABLE 1. Baseline Characteristics of Patients at Admission to the ICU

	Positive Microbiology $n = 188$	Negative Microbiology $n = 82$	p
Age, yr	63±15	65±12	0.27
Sex, male/female, n	133/55	60/22	0.79
Alcohol abuse (current or former), n (%)	47 (25)	22 (27)	0.87
Smoking habit (current or former), n (%)	95 (51)	42 (51)	>0.99
Acute Physiology and Chronic Health Evaluation II score	17±6	17±5	0.76
Simplified Acute Physiology Score	40±12	43±14	0.21
Sepsis-related Organ Failure Assessment Score	7±3	8±3	0.19
Comorbidities, n (%)			
Diabetes mellitus	37 (20)	23 (28)	0.17
Chronic renal failure	11 (6)	15 (18)	0.003
Solid cancer	33 (18)	12 (15)	0.68
Chronic heart disorders	55 (29)	35 (43)	0.044
Chronic lung disease	67 (36)	22 (27)	0.20
Chronic liver disease	31 (17)	16 (20)	0.67
Recent surgery, n (%)	94 (50)	48 (59)	0.25
Tracheostomy at admission, n (%)	20 (11)	5 (6)	0.34
Causes of ICU admission, n (%)			0.059
Postoperative	38 (20)	24 (29)	0.14
Hypoxemic lung insufficiency	27 (14)	12 (15)	>0.99
Decreased consciousness	30 (16)	3 (4)	0.008
Hypercapnic lung insufficiency	23 (12)	9 (11)	0.93
Septic shock	15 (8)	11 (13)	0.24
Multiple trauma	17 (9)	3 (4)	0.19
Nonsurgical abdominal disease	10 (5)	8 (10)	0.28
Acute coronary syndrome	7 (4)	6 (7)	0.22
Cardiac arrest	8 (4)	3 (4)	>0.99
Other	13 (7)	3 (4)	

standard methods (25). Polymicrobial pneumonia was defined when more than one PPM was identified as causative agents.

The initial empiric antimicrobial treatment was administered according to local adaptation of the American Thoracic Society/Infectious Disease Society of America guidelines (1), based on the most frequently isolated PPM and their patterns of antimicrobial sensitivity in our institution, and subsequently revised according to the microbiologic results.

VAP was diagnosed in patients with previous invasive mechanical ventilation for 48 hours or more. Patients were clustered into VAP and nonventilator ICUAP (i.e., cases who do not meet VAP criteria) (2). Early-onset pneumonia was defined as occurring within the first 4 days of hospitalization (1). The

empiric antimicrobial treatment was considered appropriate when the isolated pathogens were susceptible in vitro to at least one of the antimicrobials administrated at adequate dose (26).

The initial response to treatment was evaluated after 72 hours of antimicrobial treatment. In patients with initial non-response to treatment (27), cultures of respiratory samples and blood were obtained again, and the empiric antimicrobial treatment was revised.

Assessment of Systemic Inflammatory Response

We evaluated the serum levels of interleukin (IL)-6, IL-8, IL-10, tumor necrosis factor-alpha, C-reactive protein, and procalcitonin within the first 24 hours and the third day after
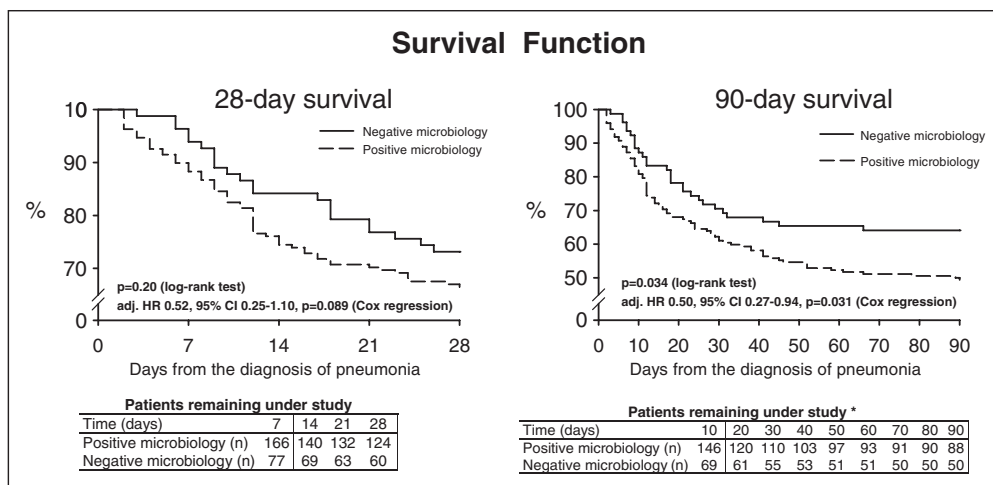
Figure 2. Kaplan-Meier curves showing the 28-day (*left*) and 90-day (*right*) survival of patients with ICU-acquired pneumonia with positive and negative microbiology. *Solid line* = negative microbiology group, *dotted line* = positive microbiology group; adj. HR = adjusted hazard ratio, 95% CI = 95% confidence interval. *Thirteen patients (7%) in the positive microbiology and four (5%) in the negative microbiology groups were lost for follow-up after days of the onset of pneumonia.

the diagnosis of pneumonia. All methods of this analysis have been recently described in details (28). We also determined mid-regional pro-adrenomedullin (MR-proADM) using a test based on the time-resolved amplification of cryptate emission technology (KRYPTOR; BRAHMS AG; Hennigsdorf, Germany). MR-proADM has an analytical detection limit of 0.08 nmol/L (normal reference range 0.33 ± 0.7 nmol/L) and a functional assay sensitivity of 0.12 nmol/L.

Data Collection
All relevant data were collected at admission and at onset of pneumonia from the medical records and bedside flow charts, including laboratory, radiologic, and microbiologic information. We calculated the Acute Physiology and Chronic Health Evaluation II (APACHE-II) (29), the Simplified Acute Physiology Score (SAPS) (30), and the Sequential Organ Failure Assessment (SOFA) (31) scores. Patients were followed until death or up to 90 days after the diagnosis of pneumonia. Septic shock (32) and acute respiratory distress syndrome (ARDS) (33) were defined according to the previously described criteria.

Outcomes Variables
The outcomes of patients with positive microbiology were compared with those of patients with negative microbiology.

TABLE 2. Characteristics of Patients at Onset of Pneumonia

	Positive Microbiology $n = 188$	Negative Microbiology $n = 82$	p
Ventilator-associated pneumonia, n (%)	135 (72)	44 (54)	0.006
Hospital stay before pneumonia, d	12 ± 13	14 ± 16	0.22
ICU stay before pneumonia, d	7 ± 9	5 ± 5	0.001
Sepsis-related Organ Failure Assessment score	7 ± 3	8 ± 3	0.35
Multilobar involvement, n (%)	80 (43)	38 (46)	0.66
Acute respiratory distress syndrome criteria, n (%)	38 (20)	23 (28)	0.21
Pleural effusion, n (%)	42 (23)	23 (28)	0.41
Shock at onset of pneumonia, n (%)	95 (51)	44 (54)	0.73
Temperature day 1, °C	36.6 ± 3.1	36.6 ± 1.5	0.90
Serum creatinine, mg/dL	1.2 ± 1	1.4 ± 1.1	0.13
Blood hemoglobin, g/L	10.4 ± 1.7	10.2 ± 1.8	0.40
White blood cell count, L^{-9}	$14,169 \pm 7,209$	$14,971 \pm 7,188$	0.40
C-reactive protein, mg/dL	15.8 ± 10.2	13.9 ± 8.7	0.14
Pao_2/Fio_2, mm Hg	199 ± 79	165 ± 73	0.001
CPIS day 1	6.6 ± 1.5	6.9 ± 1.4	0.074
CPIS day 3	5.6 ± 1.8	6.2 ± 1.8	0.016
Change in CPIS from day 1 to day 3	-0.95 ± 1.85	-0.74 ± 1.89	0.39

CPIS = Clinical Pulmonary Infection Score.

TABLE 3. Main Characteristics of Patients at Baseline and at the Onset of Pneumonia Separated According to Previous Mechanical Ventilation or Not

	Ventilator-Associated Pneumonia			Nonventilator ICU-Acquired Pneumonia		
	Positive Microbiology $n = 136$	Negative Microbiology $n = 44$	p	Positive Microbiology $n = 52$	Negative Microbiology $n = 38$	p
Age, yr	62±16	63±14	0.55	66±15	67±9	0.72
Sex, male/female, n	96/40	31/13	>0.99	38/14	29/9	0.92
Acute Physiology and Chronic Health Evaluation II score	17±6	16±5	0.36	16±5	17±5	0.34
Simplified Acute Physiology Score	41±13	44±15	0.80	36±11	40±13	0.25
Chronic renal failure	8 (6)	7 (16)	0.055	4 (8)	8 (21)	0.13
Chronic heart disorders	38 (28)	19 (43)	0.089	18 (35)	16 (42)	0.61
Chronic lung disease	42 (31)	13 (30)	>0.99	25 (48)	9 (24)	0.033
Causes of ICU admission, n (%)						
Postoperative	30 (22)	16 (36)	0.091	9 (17)	8 (21)	0.86
Hypoxemic lung insufficiency	9 (7)	5 (11)	0.34	17 (33)	7 (18)	0.20
Decreased consciousness	30 (22)	2 (5)	0.016	0 (0)	1 (0.4)	0.87
Hypercapnic lung insufficiency	9 (7)	5 (11)	0.34	14 (27)	4 (11)	0.098
Septic shock	9 (7)	6 (14)	0.21	6 (12)	5 (13)	>0.99
Multiple trauma	16 (12)	3 (7)	0.57	1 (1)	0 (0)	>0.99
Other	33 (24)	7 (16)		5 (10)	13 (34)	
ICU stay before pneumonia, d	9±8	7±5	0.079	4±7	2±4	0.063
Shock at onset of pneumonia, n (%)	71 (52)	29 (66)	0.16	24 (46)	15 (40)	0.68
Pao_2/Fio_2, mm Hg	211±80	168±72	0.002	167±66	161±75	0.72
C-reactive protein, mg/dL	15±10	13±9	0.37	19±11	15±8	0.061
Sepsis-related Organ Failure Assessment Score at onset of pneumonia	8±4	8±3	0.48	7±3	8±4	0.30
Acute respiratory distress syndrome criteria	26 (19)	14 (32)	0.12	11 (21)	9 (24)	0.98
ICU stay, d	26±20	25±23	0.67	19±13	16±17	0.30
Hospital stay, d	44±34	49±47	0.47	44±32	47±33	0.63
ICU mortality, n (%)	47 (35)	11 (25)	0.32	20 (39)	10 (26)	0.33
Hospital mortality, n (%)	60 (44)	14 (32)	0.21	24 (46)	11 (29)	0.15
28-day mortality rate, n (%)	45 (33)	12 (27)	0.59	20 (39)	9 (24)	0.21
90-day mortality rate, n (%)[a]	61 (49)	16 (39)	0.34	26 (51)	12 (32)	0.13

[a]Thirteen patients (7%) in the positive microbiology and 4 (5%) in the negative microbiology groups were lost for follow-up after days of the onset of pneumonia.

The primary outcomes were mortality in the ICU and hospital, and survival at 28 and 90 days after diagnosis of ICUAP. Secondary outcomes were the length of stay and the characteristics of patients at admission to the ICU and at onset of pneumonia.

Statistical Analysis

Categorical and continuous data are presented as number (%) and as mean ± SD (or median, interquartile range), respectively.

Categorical variables were compared with the chi-square or Fisher exact tests. Quantitative continuous variables were compared using the unpaired Student t test or the Mann-Whitney test for normally and non-normally distributed variables. The Kaplan-Meier curves were used to compare survival in the two groups.

The association of positive microbiology with patients' outcome was adjusted for variables potentially related to mortality

TABLE 4. Diagnostic Samples for Microbiologic Culture

	Positive Microbiology n = 188	Negative Microbiology n = 82	p
Sputum or tracheal aspirate,[a] n (%)	182 (97)	78 (95)	0.50
Tracheal aspirate, n (%)	168 (89)	70 (85)	
Sputum, n (%)	16 (9)	12 (14)	
Bronchoalveolar lavage, n (%)	34 (18)	24 (29)	0.058
Bronchoscopic	33 (18)	21 (26)	
Blind	1 (1)	3 (4)	
Pleural fluid culture, n (%)	24 (13)	11 (13)	>0.99
Urinary antigens, n (%)			
Streptococcus pneumoniae	74(39)	27(33)	0.39
Legionella pneumophila	79(42)	31(38)	0.61
Blood culture, n (%)	131 (70)	64 (78)	0.21

[a]Sputum or tracheal aspirates were obtained depending on whether or not patients were intubated at the onset of pneumonia. In some patients, a sample of both sputum and tracheal aspirate was processed for culture.

or survival, such as age, APACHE-II and SAPS at ICU admission, SOFA score, CPIS, and Pao_2/Fio_2 ratio at onset of pneumonia, VAP or NV-ICUAP, and unilateral or bilateral chest radiograph infiltrates. We used logistic regression analysis for ICU and hospital mortality, and Cox proportional hazard regression analysis for 28-day and 90-day survival.

A 2-sided p value less than or equal to 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 18.0.0 (Chicago, IL).

RESULTS

Patients' Characteristics

We prospectively identified 318 consecutive patients; 16 patients with NV-ICUAP were excluded because a valid LRT sample could not be obtained, 29 patients with negative microbiology in whom new antibiotics had been introduced 72 hours prior to clinical diagnosis of pneumonia, and three patients for both reasons (**Fig. 1**). Therefore, we included 270 patients: 188 (70%) with positive microbiology and 82 (30%) with negative microbiology. The characteristics of patients at admission to the ICU and at onset of pneumonia are shown in **Tables 1** to **3**.

Patients with negative microbiology had more frequently chronic renal failure, chronic heart disorders, more severe hypoxemia, and higher CPIS at day 3. Patients with positive microbiology had more frequently decreased consciousness as the cause of ICU admission and higher proportion of VAP instead of NV-ICUAP. Although the ICU stay before the onset of pneumonia was longer in patients from this group, the previous hospital stay was similar between both groups.

Microbiologic Assessment

The number of samples processed for microbiology was similar between both groups, except for a trend to a higher proportion

of BAL in patients with negative microbiology (**Table 4**). The etiology of pneumonia in the 188 patients with positive microbiology is shown in **Table 5**. The most frequently isolated pathogens were *Pseudomonas aeruginosa*, *Enterobacteriaceae*, methicillin-sensitive *Staphylococcus aureus* (MSSA), and methicillin-resistant *S. aureus* (MRSA).

Twenty-three patients (12%) in the positive microbiology group had bacteremia. The most frequently isolated pathogens in blood cultures were *Enterobacteriaceae* in nine cases, *P. aeruginosa* in four, MSSA in three, and MRSA in two cases. Bacteremia was not related to different hospital mortality or levels of inflammatory biomarkers in patients with positive microbiology.

Assessment of Systemic Inflammatory Response

The serum levels of interleukins and other inflammatory biomarkers at days 1 and 3 of pneumonia were similar between both groups (**Table 6**).

Empiric Antibiotic Treatment

The initial empiric treatment was appropriate in 162 (86%) patients with positive microbiology. Patients from the negative microbiology group received more initial antibiotics, and in those from the positive microbiology group, changes of the empiric treatment were more frequent and the total duration of treatment was longer (**Table 7**).

Outcome Variables

Although the ICU mortality rate was similar between both groups, patients with confirmed microbiology had higher hospital mortality (p =0.040; **Table 7**). After adjustment for potential confounders, positive microbiology was significantly associated with increased ICU (adjusted odds ratio [OR], 2.95; 95% confidence interval [CI], 1.17–7.44; p = 0.022) and hospital mortality (adjusted OR, 2.96; 95% CI, 1.24–7.04; p = 0.014).

TABLE 5. Etiologic Diagnosis of Pneumonia in the 188 Patients With Positive Microbiology[a]

| Pathogen	n (%)
Gram-positive bacteria	
Methicillin-sensitive *Staphylococcus aureus*	36 (19)
Methicillin-resistant *Staphylococcus aureus*	15 (8)
Streptococcus pneumoniae	9 (5)
Gram-negative bacteria	
Enterobacteriaceae[b]	60 (32)
Klebsiella species	12 (6)
Escherichia coli	16 (9)
Proteus species	6 (3)
Enterobacter species	11 (6)
Citrobacter species	4 (2)
Serratia species	10 (5)
Morganella morganii	1 (1)
Nonfermentative gram-negative bacilli	68 (36)
Stenotrophomonas maltophilia	7 (4)
Pseudomonas aeruginosa[c]	60 (32)
Acinetobacter species.	1 (1)
Moraxella catarrhalis	2 (1)
Haemophilus influenzae	8 (4)
Fungi	
Aspergillus species	6 (3)
Others	2 (1)

[a]Eighteen (10%) patients had more than one pathogen isolated.

[b]Including five cases with *E. coli* and one with *Klebsiella* species resistant to third-generation cephalosporins and quinolones; three cases with *Klebsiella* species extended-spectrum β-lactamase producing one strain of *Serratia* species resistant to aminoglycosides and third-generation cephalosporins and one strain of *E. coli* resistant to quinolones.

[c]Including eight multiresistant strains (resistant to piperacillin-tazobactam, quinolones, carbapenems, and third-generation cephalosporins); one extra-resistant strain (the prior plus aminoglycosides); two strains resistant to carbapenems, piperacillin-tazobactam, and quinolones; one strain resistant to quinolones and carbapenems; six strains resistant to quinolones; and one strain resistant to carbapenems.

In the positive microbiology group, the hospital mortality rate associated with the most frequent pathogens was 30 (50%) for *P. aeruginosa*, 23 (38%) for *Enterobacteriaceae*, 17 (47%) for MSSA, and 7 (47%) for MRSA.

Similarly, while the 28-day survival was not significantly different between both groups ($p = 0.21$; **Fig. 2**, *left*), the 90-day survival was significantly lower in patients with positive microbiology ($p = 0.034$, Fig. 2, *right*). After adjustment for potential confounders, the 28-day survival tended to be lower ($p = 0.089$), and the 90-day survival remained lower ($p = 0.031$) for patients with positive microbiology.
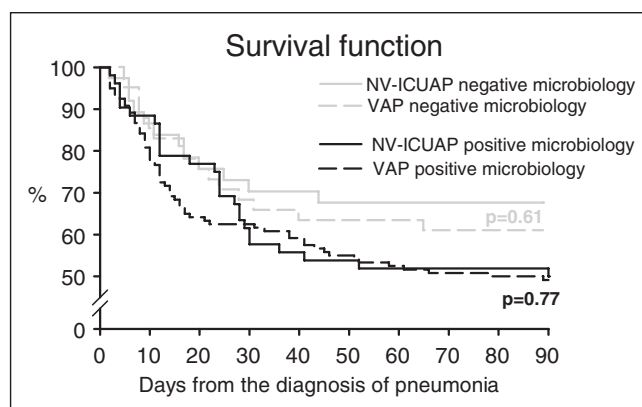
Figure 3. Kaplan-Meier curves showing the 90-day survival of patients with ICU-acquired pneumonia with positive and negative microbiology. Each group is divided into ventilator-associated pneumonia (VAP) and nonventilator ICU-acquired pneumonia (NV-ICUAP). *Black solid line* = NV-ICUAP-negative microbiology group, *black dashed line* = VAP-negative microbiology group, *gray solid line* = NV-ICUAP-positive microbiology group, *gray dashed line* = VAP-positive microbiology group.
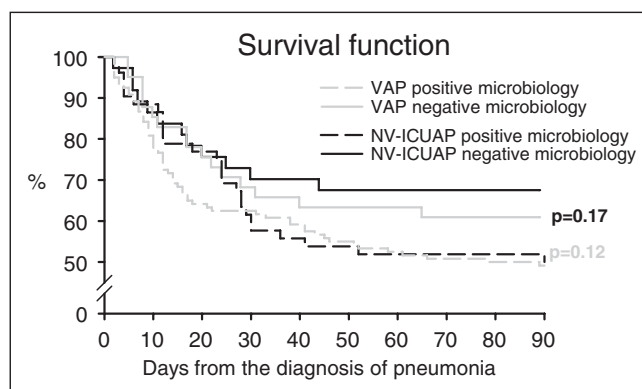
Figure 4. Kaplan-Meier curves showing the 90-day survival of patients with ICU-acquired pneumonia with ventilator-associated pneumonia (VAP) and nonventilator ICU-acquired pneumonia (NV-ICUAP) positive and negative microbiology. Each group is divided into positive and negative microbiology. *Black solid line* = NV-ICUAP-negative microbiology group, *black dashed line* = NV-ICUAP-positive microbiology group, *gray solid line* = VAP-negative microbiology group.

To avoid possible confusion, we performed two sensitivity analyses. First, we added 19 patients excluded from the study because of the absence of LRT samples. The mortality rates in the positive and negative microbiology group, respectively, were the following: ICU (36% vs 27%; $p = 0.17$), hospital (45% vs 34%; $p = 0.098$) and at 90 days (50% vs 39%; $p = 0.063$). Second, we excluded 18 patients with etiologic pathogens usually isolated in the community, such as *S. pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis*. The mortality rates in the positive and negative microbiology group, respectively, were the following: ICU (37% vs 26%; $p = 0.12$), hospital (46% vs 31%, $p = 0.028$) and at 90 days (51% vs 36%, $p = 0.041$).

Because of the higher rate of VAP in patients with positive microbiology, we have also compared separately the 90-day survival of patients with VAP and NV-ICUAP. We found no difference in the survival between patients with VAP and

TABLE 6. Serum Levels of Inflammatory Biomarkers[a]

	n[b]	Positive Microbiology	Negative Microbiology	p
IL-6 day 1, pg/mL	166	160 (40–468)	142 (64–341)	0.86
IL-6 day 3, pg/mL	138	95 (31–208)	91 (51–221)	0.83
IL-8 day 1, pg/mL	166	97 (56–193)	108 (61–197)	0.51
IL-8 day 3, pg/mL	138	79 (44–167)	101 (63–217)	0.32
TNF-α day 1, pg/mL	166	8 (5–15)	9 (5–14)	0.58
TNF-α day 3, pg/mL	138	7 (5–14)	9 (6–15)	0.074
Procalcitonin day 1, ng/mL	170	0.45 (0.11–1.58)	0.37 (0.14–1.04)	0.84
Procalcitonin day 3, ng/mL	143	0.37 (0.10–1.20)	0.34 (0.14–1.49)	0.57
MR-proADM day 1, nmol/L	169	1.34 (0.41–2.30)	1.03 (0.43–2.16)	0.57
MR-proADM day 3, nmol/L	142	1.24 (0.40–2.18)	1.09 (0.68–2.54)	0.42

IL = interleukin; TNF = tumor necrosis factor; MR-proADM = mid-regional pro-adrenomedullin.
[a]Results are given as median (interquartile range).
[b]Number of cases with blood samples processed for each inflammatory biomarker.

NV-ICUAP, both in the positive and negative microbiology groups (**Fig. 3**). Similarly, we have compared separately the 90-day survival of patients with positive and negative microbiology. Although the differences in the survival curves between positive and negative microbiology remained when patients with VAP and NV-ICUAP were analyzed separately, these differences did not reach statistical significance likely because of the smaller sample size (**Fig. 4**).

TABLE 7. Length of Stay, Antimicrobial Treatment, and Outcome Variables

	Positive Microbiology n = 188	NegativeMicrobiology n = 82	p
ICU stay, d	25±19	21±21	0.13
Hospital stay, d	45±34	48±41	0.46
No. of initial antibiotics, n (%)	2.4±0.6	2.6±0.6	0.010
Changes of empiric treatment, n (%)	112 (60)	33 (40)	0.005
Total duration of treatment, d	17±12	13±6	0.006
Adherence to guidelines, n (%)[a]	117 (63)	58 (71)	0.23
Nonresponse to treatment, n (%)	114 (61)	41 (50)	0.14
ICU mortality, n (%)	67 (36)	21 (26)	0.14
Hospital mortality, n (%)	84 (45)	25 (31)	0.040
28-day mortality rate, n (%)	65 (35)	21 (26)	0.15
90-day mortality rate, n (%)[b]	87 (50)	28 (36)	0.055
Causes of death within 90 days			0.77
Shock, n (%)	48 (57)	14 (61)	
Refractory hypoxemia, n (%)	6 (7)	2 (9)	
Order do-not-resuscitate, n (%)	16 (19)	3 (13)	
Brain anoxia, n (%)	8 (10)	1 (4)	
Others, n (%)	6 (7)	3 (13)	
Unknown, n (%)	3 (2)	5 (6)	

[a]Guidelines published in (1).
[b]Thirteen patients (7%) in the positive microbiology and 4 (5%) in the negative microbiology groups were lost for follow-up after days of the onset of pneumonia.

DISCUSSION

Despite a substantial diagnostic workup, 30% of the patients with clinical diagnosis of ICUAP remained without etiologic diagnosis. Patients with negative microbiology had more frequently chronic renal failure and cardiac disease as comorbidities, worse oxygenation, and better survival. Patients with positive microbiology had more frequently VAP instead of nonventilator ICUAP and had stayed in the ICU for longer time before the onset of pneumonia.

A similar diagnostic workup, based on the recommendations of current guidelines (1), was done in both groups, except for a trend of higher proportion of BAL samples in the negative microbiology group. This could be the consequence of previous negative cultures of respiratory samples because the decision to perform additional diagnostic tests was done by the attending physicians. The identification of causative pathogens resulted in more frequent changes of the empiric treatment, mainly because of assessment of pathogens resistant to the initial antimicrobials or out of their spectrum of activity. By contrast, the negativity of microbiologic findings resulted in shorter total duration of antimicrobial treatment. A previous study suggested that patients with clinical suspicion of VAP and negative cultures of BAL can have empiric antimicrobial therapy safely discontinued after a short period of time (34).

As described in Table 2, patients with positive microbiology had longer ICU stay before the diagnosis of pneumonia. Early-onset nosocomial pneumonia acquired in the ICU was also associated with higher rate of negative microbiology than late-onset pneumonia in a previous study (35). A possible explanation for this finding would be that, because critical illness is associated with worsening of pulmonary defenses to infection, a longer period of critical illness before the onset of pneumonia would have resulted in higher microbial burden into the lower airways.

An important issue is the lack of specificity of clinical criteria for the diagnosis of VAP or NV-ICUAP. In ventilated patients, alternative causes to the cluster of signs often attributed to VAP include alveolar edema, inflammatory exudates associated with acute infection, bleeding, aspiration, neoplastic infiltration, or fibroproliferation. Radiographic opacities can also occur from atelectasis or from pulmonary infarction of thromboembolic origin. Meduri et al (36) found that VAP was present in a minority of ICU patients with fever and pulmonary densities and that both clinical criteria and chest radiographs were of limited value. Furthermore, they found that atelectasis and congestive heart failure were common causes of pulmonary densities in patients without ARDS . In our population, the negative microbiology group had more frequently underlying renal and cardiac comorbidities and worse oxygenation. In this context, some of these cases might also represent, at least in part, fluid overload because of renal failure or congestive heart failure added to the underlying inflammatory process potentially mimicking pneumonia. Ewig et al (37) found similar results in patients with community-acquired pneumonia of unknown microbial etiology.

Negative microbiologic results in patients with suspected ICUAP might be explained by several additional reasons. First, the effects of prior antibiotic treatment. For this reason, we excluded from the analysis, those patients with new antibiotic treatment in the last 72 hrs prior to diagnosis and negative microbiology. In spite of this, we cannot discard that antibiotics administered for other reasons before the current episode could affect negatively the growth of microorganisms. Second, a low sensitivity of the LRT samples obtained. This is hard to understand because all cultured TBA samples were of good quality, and in some cases, we obtained very reliable samples such as BAL. Third, nonbacterial causes of pneumonia. It has been seen recently that *herpes simplex virus* and *cytomegalovirus* could be the potential causes of VAP (38–41). We did not search for viruses in our samples. The design of our study does not allow to distinguish between these potential explanations.

We found a better in-hospital and 90-day survival in patients with negative microbiology, despite the fact that both groups had similar baseline severity scores and organ dysfunction as shown in Tables 1 and 2. The differences in mortality or survival between both groups were less evident when shorter periods of time, that is, in the ICU or at 28 days, were considered. In critically ill patients, 90 days seems an appropriate period for assessing survival because using 28 days, some important changes on long-term will be lost (42). Although the prognostic impact of VAP in terms of attributable mortality has been recently questioned (5), the higher mortality of patients with microbiologically confirmed pneumonia could support that there exists an attributable mortality in patients with ICUAP. In contrast, a previous study observed similar 60-day mortality between patients with suspected and microbiologically confirmed VAP (43). However, the confirmed diagnosis of VAP in this study was based solely on BAL or protected specimen brush, techniques that are known to have lower sensitivity than their combination with quantitative TBAs.

The CPIS decreased similarly from day 1 to day 3 in both groups. This score was surprisingly lower at day 3 for the population with microbiologically confirmed pneumonia. However, the limited diagnostic accuracy of this score has been highlighted by several studies (44, 45).

The usefulness of procalcitonin and proinflammatory cytokines as diagnostic and prognostic markers of VAP has been recently studied. A previous study found that levels of procalcitonin were useful to exclude false-positive diagnoses of VAP (46). Similarly, IL-6 at admission was found to be an early and accurate indicator of patients at increased risk for VAP and in discriminating patients with VAP from other causes of pulmonary infiltrates (47). However, these studies were done in very small and selected populations of patients without infections at admission to the ICU. In our study, we measured serum biomarkers on the day of clinical diagnosis of pneumonia and day 3. We did not find any difference between patients with positive and negative microbiology. Similarly, crude values of procalcitonin had poor diagnostic value for VAP in a previous study (48). Patients from the present study are more representative of a real-life ICU population with causes of admission and underlying diseases often accompanied with important inflammatory response, regardless the presence or absence of pneumonia, as

previously reported (49, 50). Our findings confirm the limited usefulness of biomarkers to discriminate confirmed and non-confirmed cases of clinical diagnosis of VAP and NV-ICUAP.

Some limitations should be addressed. First, this is an observational study and therefore particularly vulnerable to confounding, despite all appropriate adjustments. Hence, we are unable to demonstrate a cause-and-effect relationship of our findings. Besides, a possible influence of the different rate of previous intubation in the mortality of both groups is not completely discarded. Second, this study was conducted in a single center and therefore, the extrapolation of the findings to other setting should be done cautiously. Third, despite using quantitative cultures to confirm ICUAP, potential false-positive and false-negative results of quantitative cultures should be acknowledged with the use of these methods. Fourth, diagnostic tests for viruses have not been systematically performed and we cannot discard that some of the microbiologically negative cases were, in fact, viral pneumonias. Fifth, despite our systematic diagnostic workup, 19 registered patients with NV-ICUAP did not have valid LRT samples collected for culture.

CONCLUSIONS

An important proportion of cases of clinical suspicion of ICUAP has no microbiologic confirmation. Negative microbiologic findings in ICUAP are associated with less frequent previous intubation, shorter duration of antimicrobial treatment, and better survival. Comprehensive future studies are needed to corroborate the presence of pneumonia in patients with suspicion of ICUAP and negative respiratory cultures.

ACKNOWLEDGMENT

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