Ventilator-Associated Pneumonia and PaO$_2$/F$_1$O$_2$ Diagnostic Accuracy: Changing the Paradigm?

Miquel Ferrer$^{1,2,*}$, Telma Sequeira$^3$, Catia Cilloniz$^{1,2,*}$, Cristina Dominedo$^4$, Gianluigi Li Bassi$^2$, Ignacio Martin-Loeches$^{2,5,*}$ and Antoni Torres$^{1,2,*}$

1. Introduction

Ventilator-associated pneumonia (VAP) remains the most frequent intensive care unit (ICU)-acquired infection [1,2]. Its incidence ranges from 9% to 27% of ventilated patients depending on diagnostic criteria [3]. VAP is associated with an increased length of stay, mortality and health care costs [4,5].
The diagnosis of VAP may be complex. Therefore, multiple efforts have been directed to overcome these difficulties and to identify the most appropriate diagnostic strategies [6], including integrative scoring systems that take into account multiple clinical data and microbiological findings.

The Clinical Pulmonary Infection Score (CPIS) score was developed to objectively diagnose VAP [6]. The score combines six variables: body temperature, white blood cell count, quantity and purulence of tracheal secretions, chest radiograph, bacterial growth in tracheal secretions and oxygenation (\(\text{PaO}_2/\text{FiO}_2 \leq 240 \text{ in mmHg}\)). However, the reported poor sensitivity and specificity of CPIS score in most studies preclude its use as an accurate diagnostic strategy [7,8]. Similarly, the Centers for Disease Control (CDC) and Prevention have proposed the inclusion of a decline in oxygenation, assessed by a \(\text{PaO}_2/\text{FiO}_2 \leq 240\), in the diagnostic criteria for VAP [9]. Current American guidelines define pneumonia as the presence of a new or progressive radiological lung infiltrate plus the clinical evidence that the infiltrate is of an infectious origin and a decline in oxygenation [2]. Indeed, the most reliable evidence of an infectious origin in patients suspected of having VAP combines these criteria with the microbiological identification of the potentially-pathogenic microorganisms (PPM) that cause pneumonia [10].

To our knowledge, no studies have comprehensively evaluated the relationship between microbiological diagnosis in populations with clinical suspicion of VAP and better or worse oxygenation, as assessed by the \(\text{PaO}_2/\text{FiO}_2\) ratio.

We hypothesized that patients with worse oxygenation would have more frequent microbiological confirmation of VAP. In order to assess the adequacy of \(\text{PaO}_2/\text{FiO}_2 \leq 240\) to help in the diagnosis of VAP, we compared the characteristics and outcomes of a real-life ICU population with suspected VAP and \(\text{PaO}_2/\text{FiO}_2 \leq \) or > 240, with special emphasis on microbiological confirmation.

2. Methods (Extended Information is Provided in Supplementary Materials)

2.1. Study Population

The study was conducted in six medical and surgical ICUs, overall comprising of 45 beds, at an 800-bed university hospital. Data were prospectively collected from 2007 to 2017. Investigators made daily rounds of each ICU. Patients older than 18 years, mechanically-ventilated for 48 h or more, with suspected VAP, were consecutively included into the study and only the first episode was analyzed. We excluded patients with severe immune-suppression [11]. The institution’s Internal Review Board approved the study (Comitè Ètic d’Investigació Clínica, registry number 2009/5427) and written informed consent was obtained from patients or their next-of-kin.

2.2. Definition of Pneumonia, Microbiologic Processing, and Antimicrobial Treatment

The clinical suspicion of pneumonia was based on 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/mm³ or leucopenia < 4000/mm³; or (3) purulent respiratory secretions) [12,13].

The microbiologic evaluation included the collection of at least one lower respiratory airways sample: tracheobronchial aspirates (TBAS) and/or bronchoscopic [14] or blind bronchoalveolar lavage (BAL) [15] if possible, within the first 24 hours of inclusion [16]. The same sampling method was performed on the third day if clinically indicated. Blood cultures and cultures from the pleural fluid were also collected if clinically justified.

Microbiologic confirmation of pneumonia was defined by the presence of at least one PPM [17] in the respiratory samples above pre-defined values (BAL > 10⁴ and TBAS > 10⁵ colony-forming units/mL, respectively, or any value if the patient was under antibiotic treatment), in pleural fluid or in blood cultures if an alternative cause of bacteremia was ruled out [18,19]. Microbiologic identification and susceptibility testing were performed by standard methods [20].

The initial empiric antimicrobial treatment was administered in all patients according to the local adaptation of guidelines [1,21], and subsequently revised according to the microbiologic results.
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 [22].

The initial response to treatment was evaluated after 72 hours of antimicrobial treatment. In patients with initial non-response to treatment [23,24], respiratory samples and blood cultures were obtained again, and the empiric antimicrobial treatment was revised.

### 2.3. Assessment of the Systemic Inflammatory Response

We evaluated the serum levels of interleukin (IL)-6, IL-8, IL-10, tumour necrosis factor-alpha (TNF-alpha), C-reactive protein (CRP), Procalcitonin (PCT), and mid-regional pro-adrenomedullin (MR-proADM) within the first 24 hours and the third day after the diagnosis of pneumonia. All methods for this analysis have been described in detail elsewhere [25,26].

### 2.4. Data Collection

All relevant data were collected at admission and at onset of pneumonia from the medical records and bedside flow charts. Patients were followed until hospital discharge, death or up to 90-days after the diagnosis of pneumonia. Septic shock [27] and acute respiratory distress syndrome (ARDS) [28] were defined according to the previously described criteria.

### 2.5. Outcomes Variables

The rate of quantitative microbiological confirmation of patients with PaO$_2$/FiO$_2$ ≤ 240 was compared with that of patients with PaO$_2$/FiO$_2$ > 240. The lowest value of PaO$_2$/FiO$_2$ on the day of VAP diagnosis was considered for patients’ group allocation. Secondary outcomes were length of stay and mortality, and 90-day survival after VAP diagnosis.

### 2.6. Statistical Analysis

Categorical and continuous data are presented as number (percentage) and as mean ± SD (or median, inter-quartile range), respectively. Categorical variables were compared with the Chi-square or Fisher’s exact tests. Quantitative continuous variables were compared using the unpaired Student’s t-test or the Mann-Whitney test for normally and not normally distributed variables, respectively. The Kaplan-Meier curves were used to compare survival in the two groups.

Univariate and multivariate logistic regression analyses were performed to assess the association of PaO$_2$/FiO$_2$ ≤ 240 or > 240 with positive microbiology. Variables that showed a significant result univariately (p < 0.10) were included in the corresponding multivariate logistic regression backward stepwise model. Adjusted odds-ratio (OR) and 95% confidence intervals (CI) were calculated.

In addition, the association between PaO$_2$/FiO$_2$ at VAP onset as continuous variable and quantitative microbiological confirmation was assessed using receiver-operator-characteristics (ROC) curve analysis. The area under the ROC curve (AUC) with 95% confidence interval (CI), and the optimal cut-off value of PaO$_2$/FiO$_2$ with sensitivity and specificity were calculated.

A two-sided p-value ≤ 0.05 was considered statistically significant. All statistical analyses were performed using SPSS 22.0.0.0 (Chicago, IL, USA).

### 3. Results

#### 3.1. Patients’ Characteristics

We prospectively identified 264 consecutive patients with suspected VAP during the study period; we excluded nine cases because PaO$_2$/FiO$_2$ at onset of pneumonia was not available. Therefore, we included 255; PaO$_2$/FiO$_2$ was ≤240 in 171 (67%) patients, and higher than this value in 84 (33%) patients (Figure 1).
Figure 1. Flow chart of the study population.

The characteristics of patients at ICU admission and pneumonia onset according \( \text{PaO}_2/\text{FiO}_2 \) are summarized in Tables 1 and 2.

Table 1. Baseline characteristics of patients at ICU admission.

|                        | \( \text{PaO}_2/\text{FiO}_2 \leq 240 \) \( n = 171 \) | \( \text{PaO}_2/\text{FiO}_2 > 240 \) \( n = 84 \) | \( p \) Value |
|------------------------|------------------------------------------------------|--------------------------------------------------|--------------|
| Age, year              | 62 ± 16                                              | 61 ± 15                                          | 0.57         |
| Sex, male/female, \( n \) | 116/55                                               | 60/24                                            | 0.56         |
| Alcohol abuse (current or former), \( n (%) \) | 41 (24)                                              | 18 (21)                                          | 0.63         |
| Smoking habit (current or former), \( n (%) \) | 90 (53)                                              | 40 (48)                                          | 0.45         |
| APACHE-II score        | 17 ± 6                                               | 19 ± 8                                           | 0.044        |
| SAPS score             | 42 ± 15                                              | 45 ± 16                                          | 0.24         |
| SOFA score             | 8 ± 3                                                | 8 ± 3                                            | 0.71         |
| Co-morbidities, \( n \) (\%) |                                                     |                                                  |              |
| Diabetes mellitus      | 41 (24)                                              | 19 (23)                                          | 0.81         |
| Chronic renal failure  | 15 (9)                                               | 5 (6)                                            | 0.43         |
| Solid cancer           | 19 (11)                                              | 10 (12)                                          | 0.85         |
| Chronic heart disorders| 54 (32)                                              | 28 (33)                                          | 0.78         |
| Chronic lung disease   | 58 (34)                                              | 30 (24)                                          | 0.10         |
| Chronic liver disease  | 27 (16)                                              | 9 (11)                                           | 0.27         |
| Recent surgery, \( n \) (\%) | 81 (47)                                              | 43 (51)                                          | 0.57         |
| Tracheotomy at admission, \( n \) (\%) | 16 (9)                                               | 11 (13)                                          | 0.37         |
| Causes of ICU admission, \( n \) (\%) |                                                      |                                                  | 0.051        |
| Post-operative         | 40 (23)                                              | 13 (16)                                          |              |
| Hypoxemic respiratory failure | 20 (12)                                             | 5 (6)                                            |              |
| Decreased consciousness| 31 (18)                                              | 17 (20)                                          |              |
| Hypercapnic respiratory failure | 16 (9)                                               | 5 (6)                                            |              |
| Septic shock           | 12 (7)                                               | 10 (12)                                          |              |
| Multiple trauma        | 12 (7)                                               | 16 (19)                                          |              |
| Non-surgical abdominal disease | 5 (3)                                               | 3 (4)                                            |              |
| Acute coronary syndrome | 10 (6)                                               | 2 (2)                                            |              |
| Cardiac arrest         | 12 (7)                                               | 9 (11)                                           |              |
| Other                  | 6 (4)                                                | 4 (5)                                            |              |

Definition of abbreviations: APACHE: Acute Physiology and Chronic Health Evaluation; ICU: Intensive Care Unit; SAPS: Simplified Acute Physiology Score; SOFA: Sepsis-related Organ Failure Assessment.
Table 2. Characteristics of patients at pneumonia onset.

|                          | PaO$_2$/FiO$_2$ ≤ 240 | PaO$_2$/FiO$_2$ > 240 | p Value |
|--------------------------|------------------------|------------------------|---------|
| **Previous antibiotics, n (%)** | 139 (81)               | 66 (79)                | 0.61    |
| **Most frequent groups, n (%):** |                        |                        |         |
| Penicillins              | 78 (46)                | 26 (31)                |         |
| Cephalosporins           | 50 (29)                | 26 (31)                |         |
| Quinolones               | 48 (28)                | 20 (24)                |         |
| Carbapenems              | 23 (13)                | 14 (17)                |         |
| Glycopeptides            | 28 (16)                | 9 (11)                 |         |
| Aminoglycosides          | 15 (9)                 | 11 (13)                |         |
| Clindamycin              | 14 (8)                 | 11 (13)                |         |
| Antifungals              | 6 (4)                  | 2 (2)                  |         |
| Hospital stay before pneumonia, days * | 7 (4–13)             | 7 (5–15)               | 0.34    |
| ICU stay before pneumonia, days * | 5 (3–9)              | 6 (4–10)               | 0.12    |
| SOFA score               | 8 ± 3                  | 7 ± 3                  | 0.004   |
| Bilateral pulmonary infiltrates, n (%) | 55 (32)             | 19 (23)                | 0.11    |
| ARDS criteria, n (%)     | 32 (19)                | 1 (1)                  | <0.001  |
| Pleural effusion, n (%)  | 56 (33)                | 18 (22)                | 0.10    |
| PaO$_2$/FiO$_2$          | 161 ± 47               | 301 ± 49               | <0.001  |
| FiO$_2$                  | 0.55 ± 0.18            | 0.41 ± 0.11            | <0.001  |
| PEEP, cmH$_2$O           | 7.4 ±3.4               | 7.8 ± 3.0              | 0.36    |
| PaCO$_2$, mmHg           | 41 ± 9                 | 39 ± 7                 | 0.066   |
| pH$_a$                   | 7.40 ± 0.09            | 7.41 ± 0.07            | 0.33    |
| Shock at onset of pneumonia, n (%) | 96 (56)             | 32 (38)                | 0.007   |
| Temperature              | 36.9 ± 1.4             | 37.0 ± 1.3             | 0.37    |
| Serum creatinine, mg/dL  | 1.3 ± 1.0              | 1.2 ± 1.2              | 0.85    |
| Blood haemoglobin, g/L   | 11 ± 2                 | 10 ± 2                 | 0.67    |
| White blood cell count, L$^{-9}$ | 14 ± 7              | 13 ± 6                 | 0.11    |
| Sodium, mEq/L            | 139 ± 6                | 141 ± 8                | 0.042   |
| Potassium, mEq/L         | 4.0 ± 0.7              | 4.1 ± 0.5              | 0.75    |
| CPIS day 1               | 7 ± 1                  | 5 ± 1                  | <0.001  |
| CPIS day 3               | 6 ± 2                  | 5 ± 2                  | <0.001  |

* Results given as median (inter-quartile range). Definition of abbreviations: ICU: Intensive Care Unit; SOFA: Sepsis-related Organ Failure Assessment; ARDS: Acute Respiratory Distress Syndrome; PaO$_2$/FiO$_2$: ratio of arterial oxygen tension to inspired oxygen fraction; CPIS: Clinical Pulmonary Infection Score.

Compared to patients with PaO$_2$/FiO$_2$ > 240, those with PaO$_2$/FiO$_2$ ≤ 240 had a lower APACHE-II score at ICU admission. However, at onset of pneumonia, patients with PaO$_2$/FiO$_2$ ≤ 240 had a higher SOFA score and CPIS, in both cases due to the lower values of PaO$_2$/FiO$_2$, presented ARDS criteria and shock more frequently, and had lower serum levels of sodium. Reasons for ICU admission were slightly different between groups ($p = 0.051$), with higher proportions of multiple trauma and septic shock among patients with PaO$_2$/FiO$_2$ > 240, and higher proportions of postoperative care and hypoxemic respiratory failure in patients with PaO$_2$/FiO$_2$ ≤ 240. The evolution of PaO$_2$/FiO$_2$ at pneumonia onset and at day 3 in both groups is shown in Figure 2.
3.2. Microbiological Aetiology

The etiologic diagnosis of patients is shown in Table 3. The number of samples processed for microbiology was similar between both groups. Pneumonia was microbiologically confirmed in 188 (74%) cases, with a lower rate of microbiological confirmation in patients with PaO$_2$/FiO$_2$ $\leq$ 240, compared with those with PaO$_2$/FiO$_2$ > 240 (117, 69% vs. 71, 85%, $p = 0.007$; odds-ratio 0.40, 95% CI 0.21 to 0.79, $p = 0.008$). The multivariate logistic regression analysis showed that PaO$_2$/FiO$_2$ $\leq$ 240 mmHg was independently associated with less microbiological confirmation of pneumonia (adjusted OR 0.37, 95% CI 0.15–0.89, $p = 0.027$). The association between PaO$_2$/FiO$_2$ and microbiological confirmation of VAP was poor, although statistically significant, with an AUC 0.645 (95% CI 0.568 to 0.725, $p = 0.040$). The optimal cut-off value of PaO$_2$/FiO$_2$ associated with microbiological confirmation of VAP was 223, with sensitivity 45% and specificity 79%.

The most frequent pathogens identified were *Pseudomonas aeruginosa*, Gram-negative enteric bacteria and *Staphylococcus aureus*, with no significant differences in the relative proportion of any pathogen among patients with microbiological diagnosis of ICUAP.
Table 3. Etiologic diagnosis of pneumonia according to PaO$_2$/FIO$_2$.

| Pathogen                              | PaO$_2$/FIO$_2 \leq 240$   | PaO$_2$/FIO$_2 > 240$ | p Value |
|---------------------------------------|----------------------------|-----------------------|---------|
| Sample processed for microbiology, n (%) | n = 171                    | n = 84                |         |
| Endotracheal aspirates                | 157 (92)                   | 79 (94)               | 0.52    |
| Bonchoalveolar lavage                 | 38 (23)                    | 21 (25)               | 0.62    |
| Blood                                 | 126 (74)                   | 58 (69)               | 0.44    |
| Pleural fluid                         | 18 (11)                    | 5 (6)                 | 0.23    |
| Positive microbiology, n (%)          | 117 (69)                   | 71 (85)               | 0.007   |
| Gram-positive bacteria, n (%)         | 37 (32)                    | 23 (32)               | 0.96    |
| MS Staphylococcus aureus              | 18 (15)                    | 17 (24)               | 0.20    |
| MR Staphylococcus aureus              | 12 (10)                    | 4 (6)                 | 0.41    |
| Streptococcus pneumonia               | 8 (7)                      | 2 (3)                 | 0.39    |
| Gram-negative enteric bacteria, n (%) | 39 (33)                    | 16 (23)               | 0.16    |
| Enterobacter spp                      | 7 (6)                      | 2 (3)                 | 0.53    |
| Klebsiella spp                        | 11 (9)                     | 6 (9)                 | 0.78    |
| Escherichia coli                      | 9 (8)                      | 2 (3)                 | 0.62    |
| Proteus spp                          | 2 (2)                      | 2 (3)                 | 0.99    |
| Citrobacter spp                       | 3 (3)                      | 3 (4)                 | 0.84    |
| Serratia spp                         | 8 (7)                      | 4 (6)                 | 0.97    |
| Morganella morganii                   | 1 (1)                      | 0 (0)                 | 0.80    |
| Non-fermentating gram-negative bacilli | 38 (32)                   | 29 (41)               | 0.32    |
| Stenotrophomonas maltophilia         | 7 (6)                      | 3 (4)                 | 0.85    |
| Pseudomonas aeruginosa                | 31 (27)                    | 26 (37)               | 0.19    |
| Other gram-negative bacteria          |                            |                       |         |
| Moraxella catarrhalis                | 2 (2)                      | 0 (0)                 | 0.71    |
| Haemophilus influenzae                | 3 (3)                      | 4 (6)                 | 0.50    |
| Fungi, n (%)                          | 2 (2)                      | 1 (1)                 | 0.66    |
| Aspergillus spp                       | 2 (2)                      | 1 (1)                 | 0.66    |
| Others, n (%)                         | 0 (0)                      | 1 (5)                 | -       |
| Polymicrobial aetiology *             | 19 (11)                    | 9 (11)                | 0.91    |

* Polymicrobial pneumonia was defined when more than one potentially-pathogenic microorganism was identified as causative agents.

3.3. Systemic Inflammatory Response

The serum levels of all inflammatory biomarkers at pneumonia onset and at day 3 are shown in Table 4. Patients with PaO$_2$/FIO$_2 \leq 240$ had higher serum levels of Procalcitonin at pneumonia onset, without any significant difference in the remaining biomarkers.

Table 4. Serum levels of inflammatory biomarkers *.

| n † | PaO$_2$/FIO$_2 \leq 240$ n = 171 | PaO$_2$/FIO$_2 > 240$ n = 84 | p Value |
|-----|----------------------------------|-----------------------------|---------|
| C-reactive protein day 1, mg/dL | 164 13 (6–21) | 81 11 (4–19) | 0.13    |
| C-reactive protein day 3, mg/dL | 149 11 (4–19) | 78 9 (5–15) | 0.078   |
| IL-6 day 1, pg/mL                | 89 168 (72–431) | 36 109 (41–229) | 0.077   |
| IL-6 day 3, pg/mL                | 75 91 (19–204) | 32 78 (33–183) | 0.98    |
| IL-8 day 1, pg/mL                | 89 108 (64–214) | 36 94 (57–137) | 0.17    |
| IL-8 day 3, pg/mL                | 75 76 (41–145) | 32 99 (63–170) | 0.15    |
| TNF-alpha day 1, pg/mL           | 89 8 (5–17) | 36 7 (5–14) | 0.73    |
| TNF-alpha day 3, pg/mL           | 75 7 (4–14) | 32 8 (5–15) | 0.61    |
| Procalcitonin day 1, ng/mL       | 90 0.45 (0.14–1.37) | 37 0.19 (0.07–0.59) | 0.037   |
| Procalcitonin day 3, ng/mL       | 78 0.30 (0.10–1.06) | 34 0.20 (0.08–0.74) | 0.57    |
| MR-proADM day 1, nmol/L          | 98 1.27 (0.33–2.22) | 40 1.16 (0.65–2.23) | 0.84    |
| MR-proADM day 3, nmol/L          | 87 1.36 (0.38–2.37) | 37 1.08 (0.61–2.04) | 0.92    |

* Results are given as median (inter-quartile range). † Number of cases with blood samples processed for each inflammatory biomarker and group. Definition of abbreviations: IL: interleukin; TNF: tumor necrosis factor; MR-proADM: mid-regional pro-adrenomedullin.
3.4. Empiric Antibiotic Treatment and Outcome Variables

Initial non-response to treatment, length of stay and ICU mortality were similar between both groups (Table 5).

| Table 5. Outcome variables. |
|-----------------------------|
|                            | \( \text{PaO}_2/\text{F}_{1\text{O}_2} \leq 240 \) | \( \text{PaO}_2/\text{F}_{1\text{O}_2} > 240 \) | \( p \) Value |
| ICU stay, days *            | 19 (12–29) | 18 (13–32) | 0.75 |
| Hospital stay, days *       | 34 (19–56) | 43 (21–56) | 0.34 |
| Non-response to treatment, \( n \) (%) | 94 (55) | 48 (57) | 0.74 |
| ICU mortality, \( n \) (%)   | 53 (31) | 18 (24) | 0.11 |
| Hospital mortality, \( n \) (%) | 71 (42) | 24 (29) | 0.044 |
| Ventilator-free days at day 28 * | 3 (0–21) | 17 (0–24) | 0.022 |
| Causes of death within 90 days: | | | |
| Shock, \( n \) (%)          | 52 (74) | 14 (58) | 0.28 |
| Refractory hypoxemia, \( n \) (%) | 4 (6) | 2 (8) | |
| Order do-not-resuscitate, \( n \) (%) | 1 (1) | 2 (8) | |
| Brain anoxia, \( n \) (%)   | 11 (16) | 6 (25) | |
| Others, \( n \) (%)         | 2 (3) | 0 (0) | |

* Results given as median (inter-quartile range). Definition of abbreviations: ICU: Intensive Care Unit.

Hospital mortality was higher, and the ventilator-free days were lower, in patients with \( \text{PaO}_2/\text{F}_{1\text{O}_2} \leq 240 \), while 90-day survival tended to be lower in this group (\( p = 0.070 \), Figure 3).

Figure 3. Survival curves at 90 days.

4. Discussion

To the best of our knowledge, this is the first study investigating the relationship between quantitative microbiological confirmation and oxygenation in a prospective cohort of patients mechanically ventilated and suspected of having VAP. We found that \( \text{PaO}_2/\text{F}_{1\text{O}_2} \leq 240 \) was associated with less microbiological confirmation in this subgroup of patients. In addition, patients with worse oxygenation had higher hospital mortality.
The diagnosis of VAP yields on clinical criteria; however, due to their potential subjectivity, other parameters has been proposed in order to better define VAP episodes [29]. Over the last years, due to an excess of antibiotic treatment, the United States Critical Care Collaborative Societies and the Department of Health and Human Services have proposed the implementation of “ventilator-associated complications” and “ventilator-associated infections” [30]. However, the use of “more objective” criteria such as chest X-ray and oxygenation has important limitations. Chest X-rays may be difficult to interpret in ICU patients due to multiple non-infectious causes such as congestive heart failure, atelectasis, prior chronic lung disease, or ARDS.

Microbiological confirmation is probably the best matching to assess VAP diagnosis quality [10]. We hypothesized that patients with more impaired oxygenation would have more frequently etiologic diagnosis and therefore they would be considered as definitive VAP episodes. Surprisingly our findings demonstrated the opposite. We previously reported that negative microbiology in patients with both community-acquired and ICU-acquired pneumonia was associated with renal and cardiac co-morbidities [31,32]. These studies suggested that 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. These conditions often exhibit more severe deterioration of oxygenation, thus possibly explaining the association of lower PaO$_2$/FiO$_2$ with less microbiological confirmation of VAP. However, lack of detection of a PPM would not preclude existence of an infection since many patients are previously treated with antibiotics. Therefore, until more specific diagnostic criteria are available, initiation of antimicrobial treatment should still be based on the clinical signs of infection together with radiographic criteria.

The use of oxygenation has been proposed in many diseases as a criterion for diagnosis. Indeed, in case of ARDS PaO$_2$/FiO$_2$ ratio is both a diagnostic and stratification tool [28,33]. Therefore, since pneumonia is the most frequent cause of ARDS [33], PaO$_2$/FiO$_2$ ratio has also been considered for pneumonia diagnosis too. Unfortunately, although PaO$_2$/FiO$_2$ might be a good parameter of severity, it might lead clinicians to over treat patients. In many situations of daily clinical practice, the use of PaO$_2$/FiO$_2$ ratio might trigger antibiotics start when they are probably not needed, and conversely patients with clear infiltrates in the X-rays but without major oxygenation derangements might preclude that the patient is not developing a VAP. In our population, 33% of suspected VAP episodes had a PaO$_2$/FiO$_2 > 240$. Therefore, the use of this threshold of PaO$_2$/FiO$_2$ in the diagnosis probably would have underestimated the incidence of VAP. This has important implications, either in the development of antimicrobial resistance due to unnecessary treatment, and an increase in mortality due to inappropriate (or none) antibiotic treatment. Using PaO$_2$/FiO$_2$ may be important to stratify patients’ severity in controlled clinical trials, and probably for an earlier or more aggressive treatment, as we report higher hospital mortality for those patients with worse oxygenation. We think, however, that patients with higher PaO$_2$/FiO$_2$ should not be excluded from clinical trials dealing with VAP.

One probable reason to point out oxygenation as a diagnosis marker is due to a wrong interpretation of CPIS as a clinical marker for VAP [6]. The CPIS can be a useful tool for severity identification when the patient has been diagnosed of VAP and can help to address patients’ response to treatment. Luna et al. using PaO$_2$/FiO$_2$ ratio could demonstrate that this was the best parameter for patients’ stratification of treatment response and a marker of clinical cure [34]. Moreover, we have previously reported that lack of improvement of PaO$_2$/FiO$_2$ was among the best predictors for adverse outcomes in ICU-acquired pneumonia [24]. This, however, faces against the use of PaO$_2$/FiO$_2$ as a diagnostic criterion, especially in some subsets of patients such as those with multiple trauma, surgery, or major burns [8]. Indeed, we found in the present study that patients with postoperative VAP episodes presented more hypoxemia compared to patients with multiple trauma. On the other hand, the recent HAP/VAP International guidelines [35] made a weak recommendation for short antibiotic courses in patients with low CPIS (i.e., 6 or less). However, this does not seem to be related to diagnosis purposes.

Diagnosis of VAP including oxygenation has not been universally accepted by many authors. In a European cohort study conducted in 465 patients with VAP, worsening oxygenation was present...
in 3 out of 4 patients [36], whilst Martin-Loeches et al. found it in two-thirds of their patients with VAP [37]. Similar to severe CAP [38], oxygenation can be and probably should be taken as a severity criteria similar to septic shock development, but it should not substitute current diagnostic parameters widely used. Several studies performed over the last decade have found that prompt antibiotic therapy was the most important determinant for improved outcomes in a general sepsis population [39]. It does not seem reasonable to add a test that based on our results would delay and probably mislead VAP diagnosis.

The current study has important strengths. It includes a long cohort of patients prospectively collected and without significant loss of follow-up due to a careful surveillance with a dedicated research team. In all the patients, blood gases were taken at the moment of diagnosis based on local protocols. Because of inaccuracy to assess the actual FiO$_2$ in non-intubated patients, we did not include cases of non-ventilator ICU-acquired pneumonia [40]. Moreover, all patients had at least one lower respiratory tract sample processed for culture at onset of pneumonia, and the frequency and type of previous antibiotic treatment is reported, without differences related to severity of oxygenation in both variables. An additional value for this study is the acquisition of inflammatory markers at the onset of pneumonia and after three days of antibiotic treatment. However, we have to acknowledge some limitations. The most important would be the single centre analysis, which might limit reproducibility of the results. However, since the same research team worked on the project during the inclusion period, the study appears reliable in terms of diagnosis and homogeneity. Second, we did not collect blood gas data at ICU admission and therefore cannot assess the evolution of oxygenation before patients were suspected of having VAP.

5. Conclusions

In conclusion, we have found that adding PaO$_2$/FiO$_2$ ratio \( \leq 240 \) to the clinical and radiographic criteria does not help in the diagnosis of VAP. PaO$_2$/FiO$_2$ ratio \( > 240 \) does not exclude this infection. Using this threshold may underestimate the incidence of VAP. Changing this paradigm might have beneficial effects on the early antibiotic treatment for VAP.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0383/8/8/1217/s1, Supplementary Data, Methods.

Author Contributions: Conception and design: M.F., A.T., T.S.; acquisition, analysis or interpretation of data: M.F., A.T., C.C., C.D., G.L.B. and I.M.-L. Drafting the manuscript for important intellectual content: M.F., A.T., C.C., C.D., G.L.B., I.M.-L.; Statistical analysis: A.T.; administrative, technical or material support: C.C., C.D., A.T.; All authors reviewed, revised, and approved the manuscript for submission; study supervision: M.F., A.T., C.C., C.D., I.M.

Funding: 2017-SGR-787: ICREA academia 2013, Juan de la Cierva 2012 (IJC-2012-14801), MEyC, PN I+D (SAF2012-33744), CibeRes (CBl06/06/0028)-ISCiii.

Acknowledgments: The results of this study have not been published elsewhere. We thank the medical and nursing staff at all the ICUs for their co-operation in the data collection. Cillóniz is the recipient of a Postdoctoral Grant (Strategic Plan for Research and Innovation in Health-PERIS 2016-2020).

Conflicts of Interest: The authors declare that they have no conflict of interest.

References

1. Kalil, A.C.; Metersky, M.L.; Klompas, M.; Muscedere, J.; Sweeney, D.A.; Palmer, L.B.; Napolitano, L.M.; O’Grady, N.P.; Bartlett, J.G.; Carratalà, J.; et al. Management of Adults with Hospital-acquired and Ventilator-associated Pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin. Infect. Dis.* 2016, 63, e61–e111. [CrossRef] [PubMed]

2. Kalanuria, A.A.; Ziai, W.; Zai, W.; Mirski, M. Ventilator-associated pneumonia in the ICU. *Crit Care* 2014, 18, 208. [CrossRef] [PubMed]

3. Timsit, J.-F.; Essaid, W.; Neuville, M.; Bouadma, L.; Mourvlier, B. Update on ventilator-associated pneumonia. *F1000Res* 2017, 6, 2061. [CrossRef] [PubMed]
4. Van Vught, L.A.; Klein Klouwenberg, P.M.C.; Spitoni, C.; Scicluna, B.P.; Wiewel, M.A.; Horn, J.; Schultz, M.J.; Nürnberg, P.; Bonten, M.J.M.; Cremer, O.L.; et al. Incidence, Risk Factors, and Attributable Mortality of Secondary Infections in the Intensive Care Unit After Admission for Sepsis. JAMA 2016, 315, 1469–1479. [CrossRef] [PubMed]

5. Metersky, M.L.; Wang, Y.; Klompas, M.; Eckenrode, S.; Bakullari, A.; Eldridge, N. Trend in Ventilator-Associated Pneumonia Rates Between 2005 and 2013. JAMA 2016, 316, 2427–2429. [CrossRef] [PubMed]

6. Pugin, J.; Auckenthaler, R.; Mili, N.; Janssens, J.P.; Lew, P.D.; Suter, P.M. Diagnosis of ventilator-associated pneumonia by bacteriologic analysis of bronchoscopic and nonbronchoscopic “blind” bronchoalveolar lavage fluid. Am. Rev. Respir. Dis. 1991, 143, 1121–1129. [CrossRef]

7. Shan, J.; Chen, H.-L.; Zhu, J.-H. Diagnostic accuracy of clinical pulmonary infection score for ventilator-associated pneumonia: A meta-analysis. Respir. Care 2011, 56, 1087–1094. [CrossRef]

8. Zilberberg, M.D.; Shorr, A.F. Ventilator-associated pneumonia: The clinical pulmonary infection score as a surrogate for diagnostics and outcome. Clin. Infect. Dis. 2010, 51 (Suppl. 1), S131–S135. [CrossRef]

9. FAQs: Pneumonia (PNEU) Events | NHSN | CDC. Available online: https://www.cdc.gov/nhsn/FAQs/FAQ-PNEU.html (accessed on 8 January 2019).

10. Chastre, J.; Fagon, J.-Y. Ventilator-associated pneumonia. Am. J. Respir. Crit. Care Med. 2002, 165, 867–903. [CrossRef]

11. Hilbert, G.; Gruson, D.; Vargas, F.; Valentino, R.; Gbikpi-Benissan, G.; Dupon, M.; Reiffers, J.; Cardinaud, J.P. Noninvasive ventilation in immunosuppressed patients with pulmonary infiltrates, fever, and acute respiratory failure. N. Engl. J. Med. 2001, 344, 481–487. [CrossRef]

12. Fábaregàs, N.; Ewig, S.; Torres, A.; El-Ebiary, M.; Ramírez, J.; de La Bellacasa, J.P.; Bauer, T.; Cabello, H. Clinical diagnosis of ventilator associated pneumonia revisited: Comparative validation using immediate post-mortem lung biopsies. Thorax 1999, 54, 867–873. [CrossRef] [PubMed]

13. Woodhead, M.; Torres, A. Definition and Classification of Community-Acquired and Nosocomial Pneumonias; European Respiratory Society Journals Ltd.: Lausanne, Switzerland, 1997.

14. Meduri, G.U.; Chastre, J. The standardization of bronchoscopic techniques for ventilator-associated pneumonia. Chest 1992, 102, 5575–5645. [CrossRef] [PubMed]

15. Kollef, M.H.; Bock, K.R.; Richards, R.D.; Heaukens, M.L. The safety and diagnostic accuracy of minibronchoalveolar lavage in patients with suspected ventilator-associated pneumonia. Ann. Intern. Med. 1995, 122, 743–748. [CrossRef] [PubMed]

16. Ruiz, M.; Torres, A.; Ewig, S.; Marcos, M.A.; Alcón, A.; Lledó, R.; Aseñor, M.A.; Maldonado, A. Noninvasive versus invasive microbial investigation in ventilator-associated pneumonia: Evaluation of outcome. Am. J. Respir. Crit. Care Med. 2000, 162, 119–125. [CrossRef] [PubMed]

17. Van Saene, H.K.F.; Petros, A.J.; Ramsay, G.; Baxby, D. All great truths are iconoclastic: Selective decontamination of the digestive tract moves from heresy to level 1 truth. Intensive Care Med. 2003, 29, 677–690. [CrossRef] [PubMed]

18. Ioanas, M.; Cavalcante, M.; Ferrer, M.; Valencia, M.; Agusti, C.; Puig de la Bellacasa, J.; Torres, A. Hospital-acquired pneumonia: Coverage and treatment adequacy of current guidelines. Eur. Respir. J. 2003, 22, 876–882. [CrossRef] [PubMed]

19. Valencia Arango, M.; Torres Martí, A.; Insastui Ordeñana, J.; Alvarez Lerma, F.; Carrasco Joaquinet, N.; Herranz Casado, M.; Tirapu Leñor, J.P.; Grupo de Estudio de la Neumonía Relacionada con Ventilación Mecánica; Grupo de Trabajo de Enfermedades Infecciosas de la SEMICYUC. Diagnostic value of quantitative cultures of endotracheal aspirate in ventilator-associated pneumonia: A multicenter study. Arch. Bronconeumol. 2003, 39, 394–399.

20. Murray, P.R.; Baron, E.J.; Joergensen, J.H.; Pfaller, M.A.; Yolken, R.H. Manual of Clinical Microbiology, 8th ed.; American Society for Microbiology: Washington, DC, USA, 2003.

21. American Thoracic Society; Infectious Diseases Society of America Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am. J. Respir. Crit. Care Med. 2005, 171, 388–416. [CrossRef]

22. Heyland, D.K.; Dodek, P.; Muscedere, J.; Day, A.; Cook, D.; Canadian Critical Care Trials Group. Randomized trial of combination versus monotherapy for the empiric treatment of suspected ventilator-associated pneumonia. Crit. Care Med. 2008, 36, 737–744. [CrossRef]
23. Ioanas, M.; Ferrer, M.; Cavalcanti, M.; Ferrer, R.; Ewig, S.; Filella, X.; de la Bellacasa, J.P.; Torres, A. Causes and predictors of nonresponse to treatment of intensive care unit-acquired pneumonia. *Crit. Care Med.* 2004, 32, 938–945. [CrossRef]

24. Esperatti, M.; Ferrer, M.; Giunta, V.; Ranzani, O.T.; Saucedo, L.M.; Li Bassi, G.; Blasi, F.; Rello, J.; Niederman, M.S.; Torres, A. Validation of predictors of adverse outcomes in hospital-acquired pneumonia in the ICU. *Crit. Care Med.* 2013, 41, 2151–2161. [CrossRef] [PubMed]

25. Ramírez, P.; Ferrer, M.; Martí, V.; Reyes, S.; Martínez, R.; Menéndez, R.; Ewig, S.; Torres, A. Inflammatory biomarkers and prediction for intensive care unit admission in severe community-acquired pneumonia. *Crit. Care Med.* 2011, 39, 2211–2217. [CrossRef] [PubMed]

26. Bello, S.; Lasierra, A.B.; Mincholé, E.; Fandos, S.; Ruiz, M.A.; Vera, E.; de Pablo, F.; Ferrer, M.; Menéndez, R.; Torres, A. Prognostic power of proadrenomedullin in community-acquired pneumonia is independent of aetiology. *Eur. Respir. J.* 2012, 39, 1144–1155. [CrossRef]

27. Singer, M.; Deutschman, C.S.; Seymour, C.W.; Shankar-Hari, M.; Annane, D.; Bauer, M.; Bellomo, R.; Bernard, G.R.; Chiche, J.-D.; Coopersmith, C.M.; et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* 2016, 315, 801–810. [CrossRef]

28. ARDS Definition Task Force; Ranieri, V.M.; Rubenfeld, G.D.; Thompson, B.T.; Ferguson, N.D.; Caldwell, E.; Fan, E.; Camporota, L.; Slutsky, A.S. Acute respiratory distress syndrome: The Berlin Definition. *JAMA* 2012, 307, 2526–2533.

29. FAQs: Ventilator-Associated (VAE) Events | NHSN | CDC. Available online: https://www.cdc.gov/nhsn/faqs/faq-vae.html (accessed on 8 January 2019).

30. Magill, S.S.; Klompas, M.; Balk, R.; Burrs, S.M.; Deutschman, C.S.; Diekema, D.; Fridkin, S.; Greene, L.; Guh, A.; Gutterman, D.; et al. Developing a new, national approach to surveillance for ventilator-associated events*. *Crit. Care Med.* 2013, 41, 2467–2475. [CrossRef] [PubMed]

31. Ewig, S.; Torres, A.; Angeles Marcos, M.; Angrill, J.; Rañó, A.; de Roux, A.; Mensa, J.; Martínez, J.A.; de la Bellacasa, J.P.; Bauer, T. Factors associated with unknown aetiology in patients with community-acquired pneumonia. *Eur. Respir. J.* 2002, 20, 1254–1262. [CrossRef]

32. Giunta, V.; Ferrer, M.; Esperatti, M.; Ranzani, O.T.; Saucedo, L.M.; Li Bassi, G.; Blasi, F.; Torres, A. ICU-acquired pneumonia with or without etiologic diagnosis: A comparison of outcomes. *Crit. Care Med.* 2013, 41, 2133–2143. [CrossRef]

33. Bellani, G.; Lafay, J.; Pham, T.; Fan, E.; Brochard, L.; Esteban, A.; Gattinoni, L.; van Haren, F.; Larsson, A.; McAuley, D.F.; et al. Epidemiology, Patterns of Care, and Mortality for Patients With Acute Respiratory Distress Syndrome in Intensive Care Units in 50 Countries. *JAMA* 2016, 315, 788–800. [CrossRef]

34. Luna, C.M.; Blanzacò, D.; Niederman, M.S.; Matarucco, W.; Baredes, N.C.; Desmery, P.; Palizas, F.; Menga, G.; Rios, F.; Apesteguia, C. Resolution of ventilator-associated pneumonia: Prospective evaluation of the clinical pulmonary infection score as an early clinical predictor of outcome. *Crit. Care Med.* 2003, 31, 676–682. [CrossRef]

35. Torres, A.; Niederman, M.S.; Chastre, J.; Ewig, S.; Fernandez-Vandellos, P.; Hanberger, H.; Kollef, M.; Bassi, G.L.; Luna, C.M.; Martin-Löeches, I.; et al. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia: Guidelines for the management of hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) of the European Respiratory Society (ERS), European Society of Intensive Care Medicine (ESICM), European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and Asociación Latinoamericana del Tórax (ALAT). *Eur. Respir. J.* 2017, 50, 1700582. [PubMed]

36. Martin-Löeches, I.; Deja, M.; Koulenti, D.; Dimopoulos, G.; Marsh, B.; Torres, A.; Niederman, M.S.; Rello, J. EU-VAP Study Investigators Potentially resistant microorganisms in intubated patients with hospital-acquired pneumonia: The interaction of ecology, shock and risk factors. *Intensive Care Med.* 2013, 39, 672–681. [CrossRef] [PubMed]

37. Martin-Löeches, I.; Povoa, P.; Rodriguez, A.; Curcio, D.; Suarez, D.; Mira, J.-P.; Cordero, M.L.; Lepeçq, R.; Girault, C.; Candeias, C.; et al. Incidence and prognosis of ventilator-associated tracheobronchitis (TAWcM): A multicentre, prospective, observational study. *Lancet Respir. Med.* 2015, 3, 859–868. [CrossRef]

38. Ferrer, M.; Travieso, C.; Cillóniz, C.; Gabarrús, A.; Ranzani, O.T.; Polverino, E.; Liapikou, A.; Blasi, F.; Torres, A. Severe community-acquired pneumonia: Characteristics and prognostic factors in ventilated and non-ventilated patients. *PLoS ONE* 2018, 13, e0191721. [CrossRef] [PubMed]
39. Martin-Loeches, I.; Levy, M.M.; Artigas, A. Management of severe sepsis: Advances, challenges, and current status. *Drug Des. Devel. Ther.* **2015**, *9*, 2079–2088. [CrossRef] [PubMed]

40. Esperatti, M.; Ferrer, M.; Theessen, A.; Liapikou, A.; Valencia, M.; Saucedo, L.M.; Zavala, E.; Welte, T.; Torres, A. Nosocomial pneumonia in the intensive care unit acquired by mechanically ventilated versus nonventilated patients. *Am. J. Respir. Crit. Care Med.* **2010**, *182*, 1533–1539. [CrossRef] [PubMed]

© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).