Usefulness of Cellular Analysis of Bronchoalveolar Lavage Fluid for Predicting the Etiology of Pneumonia in Critically Ill Patients

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Abstract

Background: The usefulness of bronchoalveolar lavage (BAL) fluid cellular analysis in pneumonia has not been adequately evaluated. This study investigated the ability of cellular analysis of BAL fluid to differentially diagnose bacterial pneumonia from viral pneumonia in adult patients who are admitted to intensive care unit.

Methods: BAL fluid cellular analysis was evaluated in 47 adult patients who underwent bronchoscopic BAL following less than 24 hours of antimicrobial agent exposure. The abilities of BAL fluid total white blood cell (WBC) counts and differential cell counts to differentiate between bacterial and viral pneumonia were evaluated using receiver operating characteristic (ROC) curve analysis.

Results: Bacterial pneumonia (n = 24) and viral pneumonia (n = 23) were frequently associated with neutrophilic pleocytosis in BAL fluid. BAL fluid median total WBC count (2,815/μL vs. 300/μL, P < 0.001) and percentage of neutrophils (80.5% vs. 54.0%, P = 0.02) were significantly higher in the bacterial pneumonia group than in the viral pneumonia group. In ROC curve analysis, BAL fluid total WBC count showed the best discrimination, with an area under the curve of 0.855 (95% CI, 0.750–0.960). BAL fluid total WBC count ≥ 510/μL had a sensitivity of 83.3%, specificity of 78.3%, positive likelihood ratio (PLR) of 3.83, and negative likelihood ratio (NLR) of 0.21. When analyzed in combination with serum procalcitonin or C-reactive protein, sensitivity was 95.8%, specificity was 95.7%, PLR was 8.63, and NLR was 0.07. BAL fluid total WBC count ≥ 510/μL was an independent predictor of bacterial pneumonia with an adjusted odds ratio of 13.5 in multiple logistic regression analysis.

Conclusions: Cellular analysis of BAL fluid can aid early differential diagnosis of bacterial pneumonia from viral pneumonia in critically ill patients.

Introduction

Severe pneumonia requiring intensive care unit (ICU) admission is associated with high rates of morbidity and mortality. Delays in the provision of adequate antimicrobial therapy have been reported to be associated with excess mortality [1–3]; therefore, rapid and accurate etiologic diagnosis of severe pneumonia is essential for successful treatment. In recent years, bronchoscopic bronchoalveolar lavage (BAL) has been established as a useful technique for collecting lower respiratory tract specimens from the alveolar level, and can thus be used to accurately define the causative organisms of pneumonia [4–6]. However, a conventional culture usually takes at least a few days, and microbiological yield is often compromised by prior empirical usage of antimicrobial agents. In addition, identification of viruses and atypical organisms requires a separate etiologic work-up.

Cellular analysis of BAL fluid, including total and differential cell counts and the CD4⁺:CD8⁺ T-lymphocyte ratio, is useful for the diagnosis of various interstitial lung diseases [7–9]. Under an appropriate clinical setting, BAL fluid analysis can provide highly suggestive or even diagnostic information for specific interstitial lung diseases in the absence of a lung biopsy [10]. However, only a
few previous studies with limited patient populations [11–13] have evaluated the role of cellular analysis of BAL fluid in patients with suspected pneumonia. Most of these studies focused on the differential diagnosis of pneumonia from non-infectious pulmonary diseases, not on the prediction of pneumonia etiology. BAL fluid analysis can be performed within several hours. Therefore, such analysis would be useful for guiding early treatment if it could predict the etiology of pneumonia, similar to the role of cerebrospinal fluid cellular analysis, which can reliably differentiate among meningitis etiologies. Therefore, this study investigated whether analysis of the cellular profile of BAL fluid can predict the etiology of pneumonia in critically ill patients admitted to the medical ICU.

Methods

Study design and setting, population, and data collection

This study was based on data from a prospective observational cohort study conducted from March 2010 to May 2013. All patients admitted to the medical ICU of Asan Medical Center, a 2,700-bed tertiary care university-affiliated hospital in Seoul, Republic of Korea, with suspected severe pneumonia were prospectively identified and monitored until discharged [14]. The data collected included patient demographics, underlying diseases or conditions, illness severity scores including Acute Physiological and Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA), type of pneumonia, laboratory data including microbiological tests, length of ICU stay, and outcome. The prospectively collected data were retrospectively analyzed. This study was approved by the Institutional Review Board of Asan Medical Center and the requirement for informed consent was waived because of the observational nature of the study. All patients information was anonymized and de-identified prior to analysis.

Inclusion and exclusion criteria

Inclusion criteria were as follows: (1) patients aged ≥18 years with a clinical diagnosis of pneumonia (see below for definition), and (2) patients who underwent bronchoscopic BAL for etiologic diagnosis of pneumonia. Exclusion criteria were as follows: (1) patients in whom the pathogen was not identified, (2) patients in whom BAL fluid analysis was impossible (due to severe neutropenia or clotting of specimen) or not performed, (3) patients with a mixed infection (identification of bacteria and virus), (4) patients who were treated with antimicrobial agents for more than 24 hours before bronchoscopic BAL, (5) patients with invasive pulmonary aspergillosis, (6) patients with mycobacterial infection, and (7) patients with *Pneumocystis jirovecii* pneumonia.

Definitions

Pneumonia was defined as the presence of an acute infiltrate on a chest radiograph and at least one of the following: fever (temperature ≥38.0°C or hypothermia (temperature <35.0°C), cough, pleuritic chest pain, dyspnea, and altered breath sounds on auscultation [15]. Pneumonia was categorized as community-acquired pneumonia (CAP), healthcare-associated pneumonia (HCAP), or hospital-acquired pneumonia (HAP), as defined previously [16,17].

Bronchoscopic BAL and BAL fluid processing and analysis

Fiberoptic bronchoscopy with BAL was performed following a standardized protocol as previously described [14]. Briefly, BAL was performed by instillation of three consecutive aliquots of sterile saline solution (20–30–30 ml) into the bronchial tree at the area that was most abnormal on the chest radiography. The right middle lobe or lingual segment was chosen in patients with bilateral diffuse infiltration. BAL fluid that was first retrieved was discarded, and BAL fluid that was subsequently retrieved was collected. The total cell count was determined using a hemocytometer. The corresponding amount of BAL fluid for 10<sup>3</sup> cells was centrifuged onto a microscope slide using a Thermo Shandon Cytopsin (Thermo Fisher Scientific Inc., Waltham, MA, USA), at 500 rpm for 5 minutes at room temperature. The slide was air-dried and stained with Wright-Giemsa stain. Differential cell counts that included percentages of neutrophils, lymphocytes, alveolar macrophages, and eosinophils were determined.

Microbiological Evaluation

Bacterial, fungal, and mycobacterial cultures of endotraheal aspirates and BAL fluid were performed. Respiratory viruses were tested by a multiplex reverse-transcription polymerase chain reaction (PCR) assay using a Seroplex RV13 ACE Detection kit (Seegene Inc., Seoul, Korea) and/or shell vial culture. PCR to detect *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, and *Legionella pneumophila*, and a urinary antigen test to detect *Streptococcus pneumoniae* and *L. pneumophila* serogroup 1 species were also performed.

Statistical analysis

Data were expressed as mean ± standard deviation or median and 25–75% interquartile range according to data distribution. Categorical variables were compared using the chi-square test or Fisher’s exact test as appropriate. Receiver operating characteristic (ROC) curves were constructed to determine the performances of BAL fluid cellular components, serum procalcitonin concentration, and C-reactive protein concentration for predicting bacterial pneumonia. Youden’s Index (sensitivity + specificity-1) [18] was used to select the optimal cutoff points of the ROC curve. Area

![Figure 1. Enrollment process for patients admitted to the medical intensive care unit due to pneumonia, with reasons for exclusion.](doi:10.1371/journal.pone.0097346.g001)
under the curve (AUC), sensitivity, specificity, positive likelihood ratio and negative likelihood ratio were calculated. For positive- and negative predictive values, the prevalence of bacterial pneumonia in severe pneumonia patients admitted to the medical ICU was assumed to be 35.9%, based on our previous study [14]. Multivariable logistic regression analysis was used to identify independent predictors of bacterial pneumonia. Variables with \( P \) values less than 0.2 in the univariate analysis were included in the multivariate analysis. The correlation between BAL fluid white blood cell (WBC) count and APACHE II score was determined by calculating Pearson’s correlation coefficient. Significance was accepted at \( P \leq 0.05 \). All tests were performed using SPSS (version 18.0; SPSS, Inc.) and GraphPad Prism (version 5; GraphPad, Inc.) software.

**Results**

**Study population**

Figure 1 shows the patient enrollment process and the reasons for exclusion. During the study period, 359 adult patients with
pneumonia underwent bronchoscopic BAL (67 with CAP, 159 with HCAP, and 133 with HAP). Of these patients, 100 were excluded because the pathogen was not identified, 42 were excluded because BAL fluid analysis was not possible (due to severe neutropenia or specimen clotting) or not performed, 52 were excluded because two or more types of pathogens were identified, and 100 were excluded because they received antimicrobial therapy for more than 24 hours before bronchoscopic BAL. Ten patients with Pneumocystis jirovecii pneumonia, 5 patients with invasive pulmonary aspergillosis, and 3 patients with mycobacterial pneumonia were also excluded. Finally, 47 patients (24 with bacterial pneumonia and 23 with viral pneumonia) were included.

**Patient characteristics**

The characteristics of the 47 patients are shown in Table 1. Thirty-two patients (68.1%) were men and the mean age was 62.1 years. Structural lung disease was the most common underlying disease (29.8%), followed by diabetes mellitus (19.0%), and hematologic malignancy/solid cancer (both 12.8%). Sixteen patients (34.0%) had CAP, 25 (53.2%) had HCAP, and 6 (12.8%) had HAP. Most baseline characteristics did not significantly differ between the bacterial pneumonia and viral pneumonia groups. By contrast, mean APACHE II (27.0±6.8 vs. 20.8±5.3, P=.002) and SOFA (11.3±3.7 vs. 7.6±3.2, P=.001) scores were significantly higher in the bacterial pneumonia group than in the viral pneumonia group. However, mortality rates, including 28-day mortality, did not significantly differ between the groups.

**Pathogens**

Pathogens that were identified in 47 patients are summarized in Table 2. Twenty-eight bacterial pathogens were identified in 24 patients. In four patients, two different bacteria were identified (S. pneumoniae + H. influenzae, S. aureus + K. pneumoniae, E. coli + E. cloacae, and P. mirabilis + P. stuartii). Twenty-six viruses were identified in 23 patients. In three patients, two different viruses were identified (influenza virus A + rhinovirus, influenza virus A + respiratory syncytial virus B, and rhinovirus + human coronavirus OC43/HKU1).

| Group                        | Pathogen                                  | Number |
|------------------------------|--------------------------------------------|--------|
| Bacterial pneumonia, (n = 24)* | Staphylococcus aureus                      | 5      |
|                              | Legionella pneumophila                     | 4      |
|                              | Streptococcus pneumoniae                  | 3      |
|                              | Haemophilus influenzae                    | 1      |
|                              | Mycoplasma pneumoniae                     | 1      |
|                              | Enteric gram-negative bacilli             | 8      |
|                              | Escherichia coli                          | 4      |
|                              | Klebsiella pneumoniae                     | 3      |
|                              | Enterobacter cloacae                      | 1      |
|                              | Proteus mirabilis                         | 1      |
|                              | P. stuartii                               | 1      |
|                              | Non-fermenting gram-negative bacilli      | 4      |
|                              | Acinetobacter baumannii                   | 3      |
|                              | Pseudomonas aeruginosa                    | 1      |
| Viral pneumonia, (n = 23)**   | Rhinovirus                                | 11     |
|                              | Influenza virus                           | 6      |
|                              | Influenza A                               | 5      |
|                              | Influenza B                               | 1      |
|                              | Respiratory syncytial virus               | 3      |
|                              | Respiratory syncytial virus A             | 2      |
|                              | Respiratory syncytial virus B             | 1      |
|                              | Parainfluenza virus                       | 2      |
|                              | Type 3                                    | 1      |
|                              | Type 2                                    | 1      |
|                              | Human coronavirus OC43/HKU1               | 2      |
|                              | Human metapneumovirus                     | 2      |
|                              | Bocavirus                                 | 1      |

Table 2. Identities of pathogens in patients with pneumonia.

Data are presented as the number (percentage) of patients.

*Twenty-eight bacterial pathogens were identified in 24 patients. In four patients, two different bacteria were identified (S. pneumoniae + H. influenzae, S. aureus + K. pneumoniae, E. coli + E. cloacae, and P. mirabilis + P. stuartii)

**| Twenty-six viruses were identified in 23 patients. In three patients, two different viruses were identified (influenza virus A + rhinovirus, influenza virus A + respiratory syncytial virus B, and rhinovirus + human coronavirus OC43/HKU1). doi:10.1371/journal.pone.0097346.t002
had two or more positive tests. Twenty-six viruses were identified in 23 patients. In three patients, two different viruses were identified. Rhinovirus was the most common virus (n = 11), followed by influenza virus (n = 6), and respiratory syncytial virus (n = 3). Viruses were identified from BAL fluid specimens in 18 patients and from nasopharyngeal specimens in 13 patients. Viruses were detected in both in BAL fluid and nasopharyngeal samples in 8 patients.

**Cellular profiles of BAL fluid**

Cellular BAL fluid profiles and distributions of BAL fluid cell counts in the two groups are shown in Table 3 and Figure 2. Detailed data of each patient are summarized in Table S1. The median total WBC count (2,815/μL vs. 300/μL, P < 0.001), percentage of neutrophils (80.5% vs. 54.0%, P = 0.02), and absolute neutrophil count (2,661/μL vs. 204/μL, P < 0.001) of BAL fluid were significantly higher in the bacterial pneumonia group than in the viral pneumonia group. The median serum procalcitonin concentration was also higher in the bacterial pneumonia group than in the viral pneumonia group (1.9 ng/ml vs. 0.3 ng/ml, P = 0.001) of BAL fluid. The procalcitonin concentration was also higher in the bacterial pneumonia group than in the viral pneumonia group (1.9 ng/ml vs. 0.3 ng/ml, P = 0.001). The diagnostic values of BAL fluid cellular components were better than those of serum procalcitonin concentration (AUC = 0.705, 95% CI; 0.554–0.855) and C-reactive protein concentration (AUC = 0.645, 95% CI; 0.487–0.803).

**Table 3. Bronchoalveolar lavage total and differential cell counts (%) in patients with pneumonia.**

|                            | Bacterial pneumonia (n = 24) | Viral pneumonia (n = 23) | P-value | Area under the ROC (95% confidence interval) |
|-----------------------------|------------------------------|--------------------------|---------|--------------------------------------------|
| Total WBC count, cell/μL    | 2,815 (645–6,163)            | 300 (130–500)            | <0.001  | 0.855 (0.750–0.960)                         |
| Neutrophils, %              | 80.5 (69.3–92.8)             | 54.0 (42.0–84.0)         | 0.02    | 0.701 (0.550–0.852)                         |
| Lymphocytes, %              | 4.0 (1.0–8.0)                | 8.0 (4.0–12.0)           | 0.02    | 0.305 (0.154–0.456)                         |
| Macrophages, %              | 12.0 (3.3–22.5)              | 20.0 (9.0–41.0)          | 0.04    | 0.324 (0.168–0.480)                         |
| Neutrophils count, cell/μL  | 2,661 (344–5636)             | 204 (48–480)             | <0.001  | 0.837 (0.724–0.950)                         |
| Lymphocytes count, cell/μL  | 61 (17–274)                  | 25 (11–58)               | 0.08    | 0.651 (0.487–0.816)                         |
| Macrophages count, cell/μL  | 144 (99–624)                 | 62 (18–160)              | 0.004   | 0.743 (0.600–0.886)                         |
| Serum procalcitonin concentration, ng/ml | 1.9 (0.2–9.6) | 0.3 (0.1–1.9) | 0.02   | 0.705 (0.554–0.855)                         |
| Serum C-reactive protein concentration, mg/dL | 20.3 (10.1–29.6) | 14.9 (5.9–23.9) | 0.09   | 0.645 (0.487–0.803)                         |

ROC = receiver operating characteristic curve. WBC = white blood cell.
Data are presented as median (interquartile range).

**Diagnostic performances of BAL fluid cellular components, serum procalcitonin concentration, and serum C-reactive protein concentration for the prediction of bacterial pneumonia**

The ability of BAL fluid cellular analysis to distinguish between bacterial pneumonia and viral pneumonia was assessed using ROC analysis (Table 3 last column and Figure 3). Total WBC count yielded the largest area under the ROC curve (AUC = 0.835, 95% confidence interval [CI] 0.750–0.960; P < 0.001), followed by neutrophil count (AUC = 0.837, 95% CI; 0.724–0.950, P < 0.001), and percentage of neutrophils (AUC = 0.701, 95% CI; 0.550–0.832, P = 0.02). The diagnostic values of BAL fluid cellular components were better than those of serum procalcitonin concentration (AUC = 0.705, 95% CI; 0.554–0.855) and C-reactive protein concentration (AUC = 0.645, 95% CI; 0.487–0.803).

The sensitivities, specificities, positive predictive values, negative predictive values, positive likelihood ratios, and negative likelihood ratios are summarized in Table 4. When the cutoff value of BAL fluid total WBC count was ≥510/μL, which was selected using Youden’s Index, specificity was 78.3% (95% CI; 67.9–93.2), sensitivity was 83.3% (95% CI; 71.4–96.9), positive likelihood ratio was 68.2% (95% CI; 49.2–82.6), negative predictive value was 98.9% (95% CI; 77.0–95.5), positive likelihood ratio was 3.83 (95% CI; 1.80–8.17), and negative likelihood ratio was 0.21 (95% CI; 0.08–0.52). A combination of BAL fluid total WBC count ≥510/μL or serum procalcitonin concentration ≥0.71 ng/mL had a sensitivity of 95.8% (95% CI; 81.6–99.8) and a negative likelihood ratio of 0.07 (95% CI; 0.003–0.40), whereas BAL fluid total WBC count ≥510/μL and serum C-reactive protein concentration ≥2.61 mg/dL had specificity of 95.7% (95% CI; 81.6–99.8) and a positive likelihood ratio of 8.63 (95% CI, 1.31–180.96).

When a cutoff value of BAL fluid total WBC count ≥510/μL was applied to 100 pathogen-identified patients who had received antimicrobial agent for more than 24 hours prior to bronchoscopic BAL (Figure 1), 44 had bacterial pneumonia, 54 had viral pneumonia, and 2 had invasive pulmonary aspergillosis. Of the 98 patients with bacterial pneumonia or viral pneumonia, the median duration of antimicrobial therapy before bronchoscopic BAL was 5 days (interquartile range, 3–9 days). The median total WBC count (395/μL vs. 200/μL, P = 0.52) and percentage of neutrophils (69.0% vs. 67.0%, P = 0.26) were not significantly different between these two groups. Figure S1 shows the changes recorded in the BAL fluid total WBC count and percentage of neutrophils according to the duration of exposure to antimicrobial agents.
Figure 2. Distributions of bronchoalveolar lavage (BAL) fluid total white blood cell (WBC) count, BAL fluid percentage of neutrophils, serum procalcitonin concentration, and serum C-reactive protein concentration. (A) total WBC count in BAL fluid, (B) percentage of neutrophils in BAL fluid, (C) serum procalcitonin concentration, and (D) serum C-reactive protein concentration. Horizontal bars indicate median values.
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Discussion

This study analyzed the usefulness of cellular analysis of BAL fluid for predicting the etiology of pneumonia in critically ill adult patients. Neutrophilic pleocytosis in BAL fluid was frequently found in patients with bacterial- and viral pneumonia. The degree of pleocytosis, which was higher in the bacterial pneumonia, was useful for differential diagnosis of bacterial pneumonia. Total WBC count had the best diagnostic accuracy for predicting bacterial pneumonia, and its diagnostic performances was better than those of serum procalcitonin and C-reactive protein concentrations. Combinations of BAL fluid total WBC count, serum procalcitonin concentration, and serum C-reactive protein concentration provided the best diagnostic yields. The data suggest that cellular analysis of BAL fluid is a rapid and useful technique for differentiating bacterial pneumonia from viral pneumonia, and can be used to direct early appropriate treatment.

Information about the role of cellular profiles of BAL fluid for differential diagnosis of bacterial pneumonia in adult patients is limited. Stolz et al [11]. evaluated potential markers of bacterial infection in a cohort of immunocompromised patients with pulmonary complications. They reported that the percentage of neutrophils in BAL fluid and the serum procalcitonin concentration are independent predictors of bacterial infection. They suggested that the optimal cutoff value of the percentage of neutrophils in BAL fluid is 15% (sensitivity 84%; specificity 77%), which is much lower than the cutoff value in the current study. Sternberg et al.[13] investigated the usefulness of BAL in assessing pneumonia in renal transplant patients, and suggested that the optimal cutoff value of the percentage of neutrophils in BAL fluid is >20% for predicting bacterial pneumonia. However, neither of these previous studies included patients with severe pneumonia caused by respiratory viruses alone, and both compared BAL findings between patients with bacterial pneumonia and those with non-infectious diseases. In the current study, patients with viral pneumonia were included by using the newly developed multiplex respiratory virus RT-PCR. This showed that cases of viral pneumonia were frequently associated with neutrophilia in BAL fluid (median 54.0%). We speculate that this underlies why the optimal cutoff value of percentage of neutrophils in BAL fluid for predicting bacterial pneumonia is much higher in the current study (64%, Table 4) than in previous studies. Several authors of the current study previously investigated the diagnostic utility of soluble triggering receptor expressed on myeloid cells-1 (sTREM-1) in BAL fluid of various patient populations with bilateral lung infiltrates. A cutoff value of ≥60% neutrophils in BAL fluid is useful for differential diagnosis of bacterial or fungal pneumonia from other causes of pneumonia or non-infectious diseases (AUC = 0.77, 95% CI: 0.54–0.84, P = 0.001) [12]. In comparison to this previous study, the current study did not include patients with non-infectious diseases or fungal pneumonia, included much more cases of severe viral pneumonia, and analyzed the counts of various cell types.

Among the currently available inflammatory markers, serum procalcitonin is one of the best indicators of bacterial infections, including lower respiratory tract infections [19]. The usefulness of serum procalcitonin measurements has been validated in the diagnosis, severity assessment, and follow-up of patients with lower respiratory tract infections [20–22]. In the current study, the AUC of serum procalcitonin concentration for predicting bacterial pneumonia (AUC = 0.705) was smaller than those of total WBC (AUC = 0.855) and neutrophil (AUC = 0.837) counts. The combination of BAL fluid WBC counts and serum procalcitonin concentration tended to improve the diagnostic accuracy of the ROC model. This indicates that combinations of these markers can be useful to rule-out (BAL fluid total WBC count ≥510/μL or serum procalcitonin concentration ≥0.71 ng/mL with a sensitivity of 95.8% and negative likelihood ratio of 0.068) or rule-in (BAL fluid total WBC count ≥510/μL and serum C-reactive protein concentration ≥26.1 mg/dl with a specificity of 95.7% and positive likelihood ratio of 8.63) bacterial pneumonia. Diagnostic accuracy could be further improved if BAL fluid cellular profiles...
Table 4. Accuracy of bacterial pneumonia diagnosis in patients with pneumonia.

| Predictor | Sensitivity | Specificity |
|-----------|-------------|-------------|
|           | n/N         | Percentile (95% CI) | n/N         | Percentile (95% CI) | PPV (95% CI)* | NPV (95% CI)* | PLR (95% CI) | NLR (95% CI) |
| Total WBC count $\geq 510$ cell/$\mu$L<sup>a</sup> | 20/24 | 83.3% (67.9–93.2) | 18/23 | 78.3% (62.2–88.6) | 68.2% (49.2–82.6) | 89.3% (77.0–95.5) | 3.83 (1.80–8.17) | 0.21 (0.08–0.52) |
| Neutrophils $\geq 64%$<sup>b</sup> | 19/24 | 79.2% (63.5–90.9) | 13/23 | 76.5% (40.2–68.8) | 50.5% (38.0–62.9) | 82.9% (67.3–92.0) | 1.82 (1.06–2.92) | 0.37 (0.13–0.91) |
| Serum procalcitonin concentration $\geq 0.7$ ng/ml<sup>b</sup> | 17/24 | 70.8% (54.8–83.8) | 15/23 | 65.2% (48.5–78.7) | 53.3% (38.1–67.9) | 80.0% (66.7–88.9) | 2.04 (1.06–3.94) | 0.45 (0.21–0.93) |
| Serum C-reactive protein concentration $\geq 26.1$ mg/dl<sup>bc</sup> | 10/24 | 41.7% (27.1–50.7) | 20/23 | 87.0% (71.7–96.3) | 64.2% (36.0–85.1) | 72.7% (64.7–79.5) | 3.19 (0.96–13.83) | 0.59 (0.49–0.65) |
| Total WBC count $\geq 510$ cell/$\mu$L and serum procalcitonin concentration $\geq 0.71$ ng/ml | 14/24 | 58.3% (42.7–68.9) | 19/23 | 82.6% (66.3–93.6) | 65.3% (42.0–83.0) | 78.0% (68.0–85.5) | 3.35 (1.27–10.84) | 0.50 (0.33–0.86) |
| Total WBC count $\geq 510$ cell/$\mu$L or serum serum procalcitonin concentration $\geq 0.71$ ng/ml | 23/24 | 95.8% (81.6–99.8) | 14/23 | 60.9% (46.0–65.0) | 57.8% (45.0–70.0) | 96.3% (78.8–99.5) | 2.45 (1.51–2.85) | 0.07 (0.003–0.40) |
| Total WBC count $\geq 510$ cell/$\mu$L and Serum C-reactive protein concentration $\geq 26.1$ mg/dl | 9/24 | 37.5% (24.1–41.4) | 22/23 | 95.7% (81.6–99.8) | 82.9% (39.9–97.2) | 73.2% (66.5–79.0) | 8.63 (1.31–180.96) | 0.65 (0.59–0.93) |
| Total WBC count $\geq 510$ cell/$\mu$L or Serum C-reactive protein concentration $\geq 11.94$ mg/dl | 21/24 | 87.5% (72.2–96.4) | 16/23 | 69.6% (53.6–78.8) | 61.7% (46.0–75.3) | 90.9% (76.9–96.7) | 2.88 (1.56–4.55) | 0.18 (0.05–0.52) |

Definition of abbreviations: NLR = negative likelihood ratio; NPV = negative predictive value; PLR = positive likelihood ratio; PPV = positive predictive value; WBC = white blood cell.

<sup>a</sup> Prevalence of bacterial pneumonia was assumed to be 35.9%, based on our previous study.

<sup>b</sup> Area under the curve using cutoff point selected by Youden’s index (sensitivity + specificity – 1).
Table 5. Multiple logistic-regression analysis of predictors for the diagnosis of bacterial pneumonia.

| Predictor                        | Odds ratio | 95% Confidence interval | P-value |
|----------------------------------|------------|-------------------------|---------|
| Total WBC count \( \geq 510 \) cell/\( \mu \)L | 13.5       | 2.3–80.4                | 0.004   |
| Serum procalcitonin concentration \( \geq 0.71 \) ng/ml | 2.6       | 0.4–17.7                | 0.32    |
| Serum C-reactive protein \( \geq 26.1 \) mg/dL | 5.0       | 0.5–47.1                | 0.16    |
| APACHE II score \( \geq 24.5 \) | 5.4       | 0.7–42.2                | 0.11    |
| Septic shock at admission        | 0.9       | 0.1–6.3                 | 0.91    |

APACHE = acute physiology and chronic health evaluation; WBC = white blood cell.

The results might be applied to patients who have received antimicrobial therapy more than 24 hours. That is, if BAL fluid cellular analysis shows evident pleocytosis even after antimicrobial therapy for more than 24 hours, it would be a strong suggestion for bacterial etiology.

The study has several limitations. First, the small sample size of the select critically ill patient population analyzed limits the general applicability of our findings. Moreover, since our study included critically ill patients with acute respiratory failure secondary to pneumonia who were not receiving antimicrobial therapy, our results may not be applicable to the majority of severe pneumonia patients in clinical practice. Second, the impact of antimicrobial therapy on the BAL fluid cellular profiles and other inflammation markers such as procalcitonin, remains to be further elucidated. Third, cases of invasive pulmonary aspergillosis, Pneumocystis jirovecii pneumonia, and mycobacterial pneumonia, were not included, mainly because few patients had these types of pneumonia. Fourth, patients with non-infectious causes of pulmonary infiltrates that can often mimic infectious causes, such as acute respiratory distress syndrome, cryptogenic organizing pneumonia, eosinophilic pneumonia, and drug-induced pneumonitis, were also excluded from our analyses. The inclusion of those cases may have caused a marked decrease in the specificity of our BAL fluid criteria. Finally, all the pathogens were not directly identified from BAL fluid. Some patients were included in whom pathogens were identified by other means, such as blood culture, endotracheal aspirates culture, urinary pneumococcal antigen test, and PCR from nasopharyngeal samples, as long as clinically and radiographically compatible and no other etiology was demonstrated. Therefore, patients with coincidental upper respiratory infections or colonization may have been included.

In conclusion, the data indicate that cellular analysis of BAL fluid, alone or in combination with serum procalcitonin and C-reactive protein concentrations, may rapidly provide valuable diagnostic information for the early differential diagnosis of pneumonia in critically ill adult patients.

Supporting Information

Figure S1  Changes in the bronchoalveolar lavage fluid (A) total white blood cell (WBC) count and (B) percentage of neutrophils according to the duration of exposure to antimicrobial agents (median plus interquartile range). (TIF)

Table S1  Characteristics and cellular profiles of bronchoalveolar lavage fluid in patients with pneumonia. (DOCX)
Author Contributions

Conceived and designed the experiments: SHC SBH YK. Analyzed the data: SHC SBH HS SOL YK. Wrote the paper: SHC SBH. Acquisition of data: SHC SBH HLH JWH. Contributed to critical revision of the manuscript for important intellectual content: SHK MNK JYV CML JHW YSK YK. Approved the final version of the manuscript: SHC SBH HLH SHK JWH HS SOL MNK JYV CML YSK JHW YK.

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