Low Alveolar Macrophage Function, Low IL-6, and High CD4 Cell Count Interaction in BALF of Severe Pneumonia Patients with Extubation Success

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Abstract

Background

Understanding the bronchoalveolar-lavage fluid immunopathology biomarker interactions can help clinicians decipher the pathophysiology of severe pneumonia and get better management for early extubation.

Objectives

The objectives were to assess bronchoalveolar lavage fluid biomarker interactions of severely affected lung and their association in determining the early extubation success.

Methods

In this cross-sectional study, we consecutively evaluated 137 severe pneumonia patients. Patients who fulfilled inclusion criteria will undergo early bronchoscopy. The BALF was collected from the right and left lungs. Two Respirologist and Critical Illness consultants, plus one internist, determined the location for severely affected lung. Biomarker interactions in severely affected lung were analyzed. The primary outcome was the 19-days extubation.

Results

Forty patients underwent bronchoscopy for BALF collection. The right lung was the predominant severely affected lung (28 patients). Eight patients survived and were successfully extubated within 19 days. There were significantly higher absolute CD4 + BALF cell counts (95% Confidence Interval = 9,24–49,50, p = 0,003) in the left lung and higher absolute CD4 + BALF cell counts (95% Confidence Interval = 9,00–29,75, p = 0,010) in the patients with extubation success and survived. Among all the patients with extubation success within 19 days, eight patients (100%) displayed the tendency of high CD4 levels (cutoff points median 16 cells/μL), low expression of alveolar macrophage function (cutoff points by ROC 756.5 MFI CD169), and low expression of IL-6 (cutoff points by ROC 369 pg/mg protein).

Conclusion

In severely affected lung of severe pneumonia patients with early extubation success and survived, we found biomarker interactions marked by low alveolar macrophage function, low IL-6 levels, and high CD4 levels.

Trial Registration

The study was registered at UMIN Clinical Trials Registry (UMIN-CTR) (registration number UMIN000046236), accessible at: https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000049197

Background

Pneumonia is the second leading reason for hospitalization of medicare beneficiaries and accounts for more than 600,000 medicare hospitalizations yearly. In 2015, it was estimated that 2.7 million individuals died from respiratory infections.[1] Severe community-acquired pneumonia is associated with a high mortality rate, and 16–36% of patients eventually die in a short period, despite effective antibiotic therapy.[2] Many critically ill patients are in immunocompromised condition and often undergo extubation failure.[3] Immunocompromised does not cause pathology but makes the patient prone to infection. Previous studies explained that depletion of the immune cells and mediators would cause the individual to be more prone to infectious diseases and/or aggravate the existing disease condition.[4] Severe pneumonia can be mediated by alveolar macrophage, sTREM-1, mononuclear cells, cytokines, Caspase-3, and SP-A. Each of these biomarkers (sTREM-1[5], alveolar macrophage[6,7], IL-6[8], IL-17[9], CD4[10,11], Tregs[12], SP-A[13], or Caspase-3[14]) have been studied separately/independently to mediate local inflammatory responses in severe pneumonia. It has been reported that BALF sTREM-1,[5] alveolar macrophage,[15] IL-6,[16] IL-17,[17] CD4 and Tregs,[18] SP-A,[19] and Caspase-3[20] played a crucial role in local immunopathology (innate and adaptive immune responses). Therefore, we assumed that the interaction of these mediators would orchestrate the immune response outcomes. The clinicians must understand complete pathophysiology to get better management for early extubation in severe pneumonia patients.[21] It is unclear how some patients can tolerate the infection and eventually result in extubation success, whereas others are likely to be in critical condition that leads to extubation failure and/or mortality. Previous studies have not comprehensively explained biomarker interactions of severely and less severely affected lung (the same patients).

Materials And Methods

A total of 137 hospitalized severe pneumonia patients were admitted to the Resuscitation Emergency Unit/Intensive Care Unit (REU/ICU) ward Cipto Mangunkusumo Hospital in November 2020 – January 2021. Patients who fulfilled inclusion criteria underwent bronchoscopy and collected bronchoalveolar lavage fluid (BALF). Demographic and clinical characteristics, laboratory findings, and eight BALF biomarkers (alveolar macrophage amount and function, sTREM-1, IL-6, IL-17, CD4, Tregs, Caspase-3, and SP-A) were recorded. The ethical committee has approved our study, Universitas Indonesia (Approval number: KET-171/UN2.F1/ETIK/PPM.00.02/2020), and the hospital review committee. Informed consent was conceived according to the local ethics committee and hospital review committee. Informed consent for bronchoscopy was signed by relatives (patients were intubated).

Study Design

This is a cross sectional study and research patients were recruited in REU/ICU ward, Cipto Mangunkusumo National Hospital. The targeted population is severe CAP in REU/ICU. The accessible population is severe CAP REU/ICU in Cipto Mangunkusumo National Hospital, Jakarta. The research

subject is an assessable population fulfilling research criteria selection recruited by consecutive sampling technique. The 19-days extubation success was selected based on the maximum mechanical ventilation duration reported by Gamberini *et al.*[22] Extubation failure was defined as patients being reintubated in 48 hours, re-intubation, and/or reused ventilator after successful extubation, and/or death.[23] Inclusion criteria: aged 18 years or older; severe pneumonia (IDSA/ATS 2007 criteria); can undergo bronchoscopy within 12 hours of admission to REU/ICU; receive empirical antibiotics of no more than 24 hours; and intubated within 24 hours. Exclusion criteria: acute respiratory distress syndrome (ARDS) non-infection; HIV/AIDS (confirmed by rapid anti-HIV testing); active malignancy within the last 12 months; on immunosuppressant therapy; refused to undergo bronchoscopy. Drop out criteria: unavailable ventilator; died within 1 hour after intubation.

Bronchoalveolar Lavage Fluid Collection

Bronchoalveolar lavage fluid was performed (an average of 4 hours after patients' intubation). The anesthesiologist gave intravenous midazolam and propofol to give optimum sedation during bronchoscopy. Chest radiography was performed prior to flexible bronchoscopy in order to determine severely and less severely affected lung, as discussed by the two Respirologist and Critical Illness consultants, and one internist, based on the affected lung chest imaging severity score proposed by Feng *et al.*[24]

The order of BAL suctioning was initially performed from less severely affected lung and proceeded to severely affected lung, from subsegment of the right middle lobe and lingula of the left lung. Severely and less severely affected lung BALF were analyzed separately. Bronchoalveolar lavage was performed (standard guidance[25]) by serial 20 mL fractions 0.9% of normal saline solution to a total volume of 100 mL (room temperature). Minimum of 60–70% of lavage volume was retrieved by gentle syringe suction collected to the mucus extractor in a wedge position and processed for further examination within 2 hours. Patients were observed for 1-hour post-procedure.

Bronchoalveolar Lavage Fluid Preparation

The BALF specimen containers were inserted in a sterilized medical plastic bag prior to transfer to the Integrated Laboratory of medical faculty, Universitas Indonesia. Specimen containers were gathered in a ventilated room. All specimen handling was coordinated by experienced laboratory staff with sufficient protective equipment. Specimen volume was evaluated for appearance, color, clearness, and contamination with intrabronchial blood. Bronchoalveolar lavage fluid specimens were collected to a 50 mL tube and then centrifuged at 1000 g for 10 min. Bronchoalveolar lavage fluid supernatant was separated to analyze sTREM-1, IL-6, IL-17, and SP-A and frozen at -80 ^{o}C. Bronchoalveolar lavage fluid pellet was suspended in 2 mL PBS to analyze alveolar macrophage, Tregs, and Caspase-3. For Caspase-3, the sample was frozen at -80^{o}C prior to transfer.

Bronchoalveolar Lavage Fluid Analysis

Flow cytometry was used to analyze alveolar macrophage, CD4, and Tregs. Enzyme-linked immunosorbent assay (ELISA) was used in duplicate to analyze sTREM-1 (MyBioSource ELISA kits, San Diego, USA), IL-6 and IL-17 (R&D systems quantikine ELISA kits, Minnesota, USA), SP-A (LSBio's ELISA kits, Washington, USA), and Caspase-3 (Cusabio ELISA kits, Texas, USA). Interleukin (IL)-6, IL-17, and SP-A were observed from the BALF supernatant. Caspase-3 was observed from cell pellet homogenate and was extracted using the freeze-thawing method (this process was done two times). Since BALF protein level is in the same concentration as in blood, total protein levels were used as an index of BALF dilution.[26] Bradford technique (Bio-RAD) was used to measure normalized protein in BALF supernatant or cell pellet.

Flow Cytometry BALF Analysis

The obtaining cell pellet (alveolar macrophage and Tregs) was incubated at room temperature in the dark, with monoclonal antibodies (mAbs) in 5 mL Polystyrene Tubes for 15 minutes, followed by insertion of FACS lysing solution for 15 minutes. The working panel of mAbs at five color assays used for alveolar macrophage and Tregs BALF evaluation were the following: anti-Human CD206 PE, anti-Human HLA-DR FITC, anti-Human CD11b APC, anti-Human CD45 PerCP-Cy5.5, and anti-Human CD169 BV421 (BD Biosciences, New Jersey, USA). The resulting cell pellet of CD4 was incubated at room temperature in the dark, with monoclonal antibodies (mAbs) in BD TruCount™ Tubes for 15 minutes, followed by insertion of FACS lysing solution for 15 minutes at room temperature with the following mAbs working panel: CD45+/CD3+/CD4+/CD8+ (BD Biosciences, New Jersey, USA). After being washed, specimens were acquired with a FACSCanto™ II flow cytometer (BD Biosciences, New Jersey, USA). BD FASCDiva™ software version 6.1.3 (BD Biosciences, New Jersey, USA) was used to perform the cytometric analysis.

Amount and function of alveolar macrophage were analyzed.[27] Alveolar macrophage (amount) was analyzed according to percentage macrophage cells / $CD45^+$, $HLA\text{-}DR^+$, and $CD11b^+$. Alveolar macrophage (function) was analyzed according to the MFI (mean fluorescence intensity) of $CD169^+$.

Statistical analysis

Numerical and categorical variables were reported as mean ± standard deviation or median (interquartile range 25th – 75th percentile), and percentages. Data normality was assessed based on Shapiro-Wilk test. A t-test was used to compare normal distribution variables (parametric data), and a Mann-Whitney Wilcoxon test was used to compare non-normal distribution continuous variables (non-parametric data). A Chi-square test was used for comparing categorical variables. Univariate analysis was performed for subject characteristics (demographic, clinical variables, and laboratory values), and bivariate analysis was performed for biomarker variables.

Statistically significant BALF biomarker(s) will be correlated to other BALF biomarkers in patients with early extubation success and survived. Method of cutoff points obtained using median or area under curve method. Cutoff points by ROC obtained by calculating maximal Youden index (= sensitivity +

specificity – 1). SPSS (Statistical Package for Social Science) version 26 software (IBM Corporation, Armonk, NY) was used to analyze all recorded data.

Results

Subject recruitment was started from November 2020 through January 2021. A total of 137 severe pneumonia patients were assessed for eligibility. Forty patients underwent bronchoscopy for BALF collection. Right lung was the predominant severely affected lung. Eight patients were successfully extubated and survived, as seen in Fig. 1.

In the patients with extubation success and survived (8 patients), there were 4 patients of male (50%), mean of age 62-year-old, BMI 27,9 kg/m^2, no smokers, severely affected right lung (4 patients), and length of stays 21 days. Diabetes mellitus (62,5%) and hypertension (62,5%) were the most common comorbidities. The Scoring system of APACHE II and mSOFA were 14,63 and 8, respectively. As expected, there was a significantly longer duration of hospital stays ($p < 0{,}001$) in the patients with extubation success and survived, as presented in Table 1.

Table 1
Demographic and Clinical Characteristics of the Study Population

Characteristic	All Patients (n = 40)	Extubation Success & Survived (n = 8)	Extubation Failure & Death (n = 32)	*p* Value
Gender (Male, %)	21 (52)	4 (50)	17 (53)	1
Age, year (± SD)	60 (± 10,8)	62 (± 8,8)	59 (± 11,3)	0,576
BMI (kg/m^2), (± SD)	26.7 (± 3,2)	27,9 (± 3,6)	26,4 (± 3,2)	0,262
Smoker, n (%)	8 (20)	0 (0)	8 (25)	0,173
Severely affected lung, (Right Lung, %)	28 (70)	4 (50)	24 (75)	0,211
Length of hospital stays (days), median (IQR)	8 (1–80)	21 (15–26,5)	6,5 (4–10,5)	**< 0,001**
Comorbidities				
Diabetes Mellitus, n (%)	22 (55)	5 (62,5)	17 (53)	0,709
Hypertension, n (%)	20 (50)	5 (62,5)	15 (47)	0,696
Chronic Kidney Disease, n (%)	17 (42,5)	2 (25)	15 (47)	0,428
COPD, n (%)	2 (5)	0 (0)	2 (6,25)	1
Bronchial Asthma, n (%)	2 (5)	1 (12,5)	1 (3,12)	0,364
Cardiovascular Disease, n (%)	2 (5)	1 (12,5)	1 (3,12)	0,364
Systemic Lupus Erythematosus, n (%)	1 (2,5)	0 (0)	1 (3,12)	1
Obesity, n (%)	1 (2,5)	1 (12,5)	0 (0)	0,200
Scoring System				
APACHE II	16 (12,5–21)	14,63 (± 3,42)	18,19 (± 6,23)	0,129
mSOFA	9 (8–11)	8 (8–9)	9 (8–12)	0,169
p Value < 0,05 (statistically significant)				
BMI denotes body mass index, COPD chronic obstructive pulmonary disease, APACHE acute physiology and chronic health evaluation, mSOFA modified sequential organ failure assessment				

There was significantly higher D-dimer value ($p = 0,010$) in the patients with extubation success and survived, as presented in Table 2.

Table 2
Laboratory Findings

Laboratory Findings	Total (n = 40)	Extubation Success & Survived (n = 8)	Extubation Failure & Death (n = 32)	*p* Value
Peripheral Blood				
Leukocytes (x10^3 cells/ µL)	13,09 (10,63–18,78)	13,20 (11,09–20,22)	13,02 (10,63–18,53)	0,478
Monocytes (%)	4,79 (± 0,33)	4,42 (± 1,38)	4,88 (± 2,62)	0,593
Lymphocytes (%)	9 (5,05–13,75)	11,65 (7,35–13,80)	8,95 (5,00–13,45)	0,510
C-reactive protein (mg/ liter)	125,6 (46,90–222,40)	118,60 (38,10–349,75)	125,60 (46,90–197,30)	0,826
Procalcitonin (ng/mL)	0,48 (0,23–1,26)	0,63 (0,14–2,72)	0,48 (0,27–0,85)	0,960
Albumin (g/dL)	3,19 (± 0,84)	2,93 (± 0,44)	3,25 (± 0,54)	0,140
D-dimer (µg/liter)	4025 (1400–10315)	10260 (5820–24080)	1960 (1175–6175)	**0,010**
PaO_2/FiO_2 ratio	63,6 (47,95–134,50)	94,47 (43,80–157,13)	61,25 (48,65–132,30)	0,636
Absolute CD4+ (cells/µL)	231,50 (125–356)	332 (216–608,50)	181 (115–334)	0,111
Inteleukin-6 (pg/mL)	48,80 (27,48–139,64)	47,62 (35,79–104,31)	54,49 (27,44–213,20)	0,973
Interleukin-17 (pg/mL)	11,74 (11,22–12,42)	11,82 (10,99–12,58)	11,59 (11,22–12,27)	1
p Value $p < 0,05$ (statistically significant)				
PaO_2/FiO_2 denotes the ratio of arterial oxygen partial pressure to fractional inspired oxygen				

There were significantly higher absolute CD4 + BALF cell counts (95% Confidence Interval = 9,24–49,50, $p = 0,003$) in the left lung of severe pneumonia patients, as presented in Table 3. Other BALF biomarkers (see the Supplementary Appendix A).

Table 3
Bronchoalveolar Lavage Fluid Findings in Right and Left Lung (Severely and Less Severely)

BALF Findings	Right Lung (n = 40)	Left Lung (n = 40)	*p* Value
Alveolar Macrophage (MFI CD169)	876,85 (332,25–810,5)	772,70 (335,5-880,25)	0,830
Absolute CD4+ (cells/μL)	27,83 (9–29,7)	44,83 (13,5–57,25)	**0,003**
Interleukin-6 (pg/mg protein)	376,38 (123,44–574,02)	327,72 (94,45–499,11)	0,582
p Value < 0,05 (statistically significant)			
BALF denotes bronchoalveolar lavage fluid, MFI mean fluorescence intensity			

There were significantly higher absolute CD4 + BALF cell counts (95% Confidence Interval = 9,00–29,75, *p* = 0,010) in the patients with extubation success and survived, as presented in Table 4. Other BALF biomarkers (see the Supplementary Appendix B).

Table 4
Bronchoalveolar Lavage Fluid Findings in Severely Affected Lung Based on Extubation Status

BALF Findings	Total (n = 40)	Extubation Success & Survived (n = 8)	Extubation Failure & Death (n = 32)	*p* Value
Alveolar Macrophage (MFI CD169)	396 (334,50–802)	373 (317,50–701,00)	432,50 (342,50–984,00)	0,187
Absolute CD4+ (cells/μL)	16 (9–36)	43,50 (21,00–59,00)	13,50 (8,50–22,50)	**0,010**
Interleukin-6 (pg/mg protein)	264,21 (124,77–577,570)	218,25 (121,85–265,68)	296,71 (124,77–626,45)	0,310
p Value < 0,05 (statistically significant)				
BALF denotes bronchoalveolar lavage fluid, MFI mean fluorescence intensity				

According to the microbial patterns, three patients were sterile in culture and negative PCR SARS-CoV-2. Twenty-eight patients with positive PCR SARS-CoV-2 (see the Supplementary Appendix C).

Among all the patients with extubation success within 19 days, 8 patients (100%) displayed the tendency of high CD4 levels (cutoff points median 16 cells/μL) with low expression of alveolar macrophage function (cutoff points by ROC 756.5 MFI CD169), conversely, among all the patients with extubation failure (32 patients), 26 patients (81.3%) did not show this tendency (Relative Risk: 0,188, 95% Confidence Interval = 0,09–0,386, *p* = 0,000), as seen in Fig. 2.

Among all the patients with extubation success within 19 days, 8 patients (100%) displayed the tendency of high CD4 levels (cutoff points median 16 cells/μL) with low expression of IL-6 (cutoff points by ROC 369 pg/mg protein), conversely, among all the patients with extubation failure (32 patients), 26 patients

(81.3%) did not show this tendency (Relative Risk: 0,188, 95% Confidence Interval = 0.091–0,386, p = 0,000), as seen in Fig. 3.

Among all the patients with extubation success within 19 days, 8 patients (100%) displayed the tendency of low IL-6 levels (cutoff points by ROC 396 pg/mg protein) with low expression of alveolar macrophage function (cutoff points by ROC 756.5 MFI CD169), conversely, among all the patients with extubation failure (32 patients), 20 patients (62.5%) did not show this tendency (Relative Risk: 0,375, 95% Confidence Interval = 0.240–0,587, p = 0,000), as seen in Fig. 4.

Other BALF biomarker interactions (see the Supplementary Appendix D)

Discussion

Based on sample size calculation, 1010 patients were required. Due to pandemic COVID-19 in our country, after enrollment of 40 patients, internal discussion was held, and the team members decided not to continue patients' enrollment.

Previous studies have described several predictors to determine extubation failure.[28,29] Our findings reported that the demographic, clinical characteristics, and routine lab profile were ineffective in determining extubation failure. Further analysis of immune biomarkers is required to evaluate the extubation success and survival. This study found that absolute CD4 + BALF cell counts were significantly higher in the patients with extubation success and survived (43,50 cells/μL vs 13,50 cells/μL, p = 0,001). By analyzing the interaction between biomarkers (alveolar macrophage amount and function, sTREM-1, IL-6, IL-17, CD4, Tregs, caspase-3, and SP-A), we revealed that there were BALF biomarker interactions in severe pneumonia patients with extubation success and survived (8 patients) marked by low alveolar macrophage function, low IL-6 levels, and high CD4 levels.

In terms of the patients' characteristics, no significant differences in demographic and clinical characteristics, except for the duration of hospital stays. As expectedly, patients with extubation success/survived had longer hospital stays, affecting cost-effectiveness and nosocomial infections risks. Based on the laboratory findings, there were also no significant differences, except for the D-dimer. We did not evaluate the serial observation for D-dimer in which presumably patients with extubation failure and non-survived could also be characterized by high D-dimer values in the later stages. Elevated D-dimer was associated with the worst outcomes and mortality in severe pneumonia patients,[30,31]; however, this is in contrast to our finding.

Our study found that the right lung was the most severely affected, as anatomically and structurally described.[32,33] A study of bronchoalveolar lavage efficacy between bilateral or unilateral sampling reported that unilateral sampling of right lung had 89% efficacy compared to bilateral sampling in both lungs (81.5%).[34] Therefore, due to this variance, the susceptibility of foreign particles and pathogens to enter the respiratory system will be more likely to the right bronchus, and later to the right lung.[32]

Previous studies have investigated the role of systemic inflammatory biomarkers to predict the extubation status in severe pneumonia patients.[35] However, to our knowledge, no previous studies had evaluated the local inflammatory biomarkers to guide the clinician in determining the extubation status of severe pneumonia patients, particularly in the right and left severely affected lung. Previous studies showed that sTREM-1,[5] alveolar macrophage,[15] IL-6,[16] IL-17,[17] CD4 and Tregs,[18] SP-A,[19] and Caspase-3[20] played a crucial role in local immunopathology (innate and adaptive immunity). Each of these immunopathology biomarkers (sTREM-1[5], alveolar macrophage[6,7], IL-6[8], IL-17[9], CD4[10,11], Tregs[12], SP-A[13], or Caspase-3[14]) have been studied separately/independently to mediate local inflammatory responses in severe pneumonia patients.

Our study showed that severe pneumonia patients with extubation success and survived tended to have low alveolar macrophage function with low IL-6 levels. Alveolar macrophage function and IL-6 are considered as the mediator/marker of the local innate immunity, whereas CD4 is a mediator/marker for adaptive immunity.[36,37] In previous reports, the role of alveolar macrophage function (marked by expression of CD169+) has been described to enhance pro-inflammatory responses, including the secretion of IL-6.[36] Patients with extubation success and survived showed that the severely affected lung had high CD4 levels, which indicates that the increased responds of cellular immunity (adaptive immune) will further enhance the patients' immunity to fight against the infection. The role of CD4 + T-cells has been described as an effector in lung immunity during critical conditions, and the depletion of CD4 will increase the worst outcome of the infection.[38] The role of BALF CD4 + T-cells has been investigated in patients with lung injury and reported that a higher percentage of BALF CD4 + T-cells, especially Tregs, could help patients' lung injury to resolve quickly and eventually undergo extubation success.[39] Our study also reported the levels of inflammatory biomarkers for Tregs [Foxp3 + CD25+ / CD4 (%)] in the supplementary, with no significant difference between the groups. As the Tregs are the division of CD4 + T cells, we assumed that the levels of Tregs and the absolute CD4 + T cells would likely contribute to the extubation success and survival of the patients. Based on the severity of the lung, our study showed that the right lung was the predominantly affected, marked by a lower number of Tregs and CD4 + T cells (statistically significant for CD4 + T cells), in which most of the patients with extubation failure and non-survived had the right lung as the most severely affected. Therefore, our study suggests that depletion of CD4 + T cells is associated with the extubation failure and death of the patients.

Our result demonstrates 19 days of extubation success and survived in severe pneumonia patients is mainly determined by biomarker interaction of low alveolar macrophage function, low IL-6 levels, and high CD4 levels BALF (severely affected lung), as depicted in Fig. 5. This ability should be specially performed in patients undergoing bronchoscopy for BALF biomarkers evaluation.

Our study limitation is that this study was not a multicenter study. The strength of this study includes the novel finding of research in terms biomarker interactions with early extubation success which might help to develop the new pathophysiology concept in severe pneumonia. To minimize mechanical ventilation's intervention risks in altering immune responses,[40] we performed early bronchoscopy with an average of

four hours after patients' intubation. Although all patients with extubation success and survived described the biomarker interactions (alveolar macrophage function, IL-6, and CD4), future studies are required to evaluate serial BALF biomarkers.

Conclusion

In severely affected lung of severe pneumonia patients with early extubation success and survived, we found biomarker interactions marked by low alveolar macrophage function, low IL-6 levels, and high CD4 levels.

Abbreviations

BALF
Bronchoalveolar lavage fluid
REU
Resuscitation emergency unit
ICU
Intensive care unit
sTREM-1
soluble Triggering Receptor Expressed on Myeloid Cells-1
IL
Interleukin
CD
Cluster of differentiation
Tregs
Regulatory Foxp3 + CD25 + CD4 + T cells
SP-A
Surfactant protein-A
BAL
Broncho alveolar lavage
ELISA
Enzyme-linked immunoassay
SD
Standard deviation
IQR
Interquartile range
COPD
chronic obstructive pulmonary disease
APACHE
Acute physiology and chronic health evaluation

mSOFA
modified sequential organ failure assessment
PaO2/FiO2
arterial oxygen partial pressure

Declarations

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Study approval

This study was approved by ethical committee at Medical Faculty, Universitas Indonesia (Approval number: KET-171/UN2.F1/ETIK/PPM.00.02/2020) and by hospital review committee.

Registration

The study was registered at UMIN Clinical Trials Registry (UMIN-CTR) (registration number UMIN000046236), accessible at: https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000049197

Consent to participate

Informed consent was conceptualized according to local ethics committee, and hospital review committee. Informed consent for bronchoscopy were signed by patient's family member (patients were intubated).

Consent to publish

Not Applicable.

Declaration of Interests

The authors have no conflicts of interest to declare.

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Contributions

G.S., performed material preparation, data collection and analysis. G.S., C.M.R., S.K.S., Z.A., I.R., T.L., E.H.P, K.H., H.W., contributed to the study conception and design. G.S., contributed to bronchoscopy and patients' management. G.S., N.B.F., S.F.M., wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Data Availability Statements

Our study data are available on request from the corresponding author (G.S). The data are not publicly available due to their containing information that could compromise the privacy of study patients.

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References

1. WHO. Global Health Estimates. 2016: Deaths by Cause, Age, Sex, by Country and by Region, 2000–2016. *World Health Organization 2018* (2018).
2. Ferrer M, et al. Severe community-acquired pneumonia: Characteristics and prognostic factors in ventilated and non-ventilated patients. PLoS ONE. 2018;13:e0191721. doi:10.1371/journal.pone.0191721.
3. Khalil Y, Ibrahim E, Shabaan A, Imam M, Behairy AEL. Assessment of risk factors responsible for difficult weaning from mechanical ventilation in adults. Egypt J Chest Dis Tuberculosis. 2012;61:159–66. doi:https://doi.org/10.1016/j.ejcdt.2012.10.005.
4. Reynolds JH, McDonald G, Alton H, Gordon S. B. Pneumonia in the immunocompetent patient. Br J Radiol. 2010;83:998–1009. doi:10.1259/bjr/31200593.
5. Gibot S, et al. Soluble triggering receptor expressed on myeloid cells and the diagnosis of pneumonia. N Engl J Med. 2004;350:451–8. doi:10.1056/NEJMoa031544.
6. Guillon A, et al. Pneumonia recovery reprograms the alveolar macrophage pool. JCI Insight 5, doi:10.1172/jci.insight.133042 (2020).
7. Kooguchi K, et al. Role of alveolar macrophages in initiation and regulation of inflammation in Pseudomonas aeruginosa pneumonia. Infect Immun. 1998;66:3164–9. doi:10.1128/iai.66.7.3164-3169.1998.
8. Montón C, et al. Cytokine expression in severe pneumonia: a bronchoalveolar lavage study. Crit Care Med. 1999;27:1745–53. doi:10.1097/00003246-199909000-00008.
9. Wonnenberg B, et al. IL-17A attracts inflammatory cells in murine lung infection with P. aeruginosa. Innate Immun. 2016;22:620–5. doi:10.1177/1753425916668244.
10. Bielosludtseva K. T. P., T. Kyreeva CD4 as a predictor of systemic inflammatory response and death at severe community-acquired pneumonia. Eur Respir J. 2013;42:4366.
11. Park H, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol. 2005;6:1133–41. doi:10.1038/ni1261.
12. Neill DR, et al. T regulatory cells control susceptibility to invasive pneumococcal pneumonia in mice. PLoS Pathog. 2012;8:e1002660. doi:10.1371/journal.ppat.1002660.
13. Mukherjee S, et al. Surfactant protein A modulates induction of regulatory T cells via TGF-β. J Immunol. 2012;188:4376–84. doi:10.4049/jimmunol.1101775.

14. Kosai K, et al. Increase of apoptosis in a murine model for severe pneumococcal pneumonia during influenza A virus infection. Jpn J Infect Dis. 2011;64:451–7.
15. Knapp S, et al. Alveolar macrophages have a protective antiinflammatory role during murine pneumococcal pneumonia. Am J Respir Crit Care Med. 2003;167:171–9. doi:10.1164/rccm.200207-698OC.
16. Paats MS, et al. Local and systemic cytokine profiles in nonsevere and severe community-acquired pneumonia. Eur Respir J. 2013;41:1378–85. doi:10.1183/09031936.00060112.
17. Orlov M, Dmyterko V, Wurfel MM, Mikacenic C. Th17 cells are associated with protection from ventilator associated pneumonia. PLoS ONE. 2017;12:e0182966. doi:10.1371/journal.pone.0182966.
18. Adamzik M, et al. An increased alveolar CD4 + CD25 + Foxp3 + T-regulatory cell ratio in acute respiratory distress syndrome is associated with increased 30-day mortality. Intensive Care Med. 2013;39:1743–51. doi:10.1007/s00134-013-3036-3.
19. Baughman RP, Sternberg RI, Hull W, Buchsbaum JA, Whitsett J. Decreased surfactant protein A in patients with bacterial pneumonia. Am Rev Respir Dis. 1993;147:653–7. doi:10.1164/ajrccm/147.3.653.
20. Du X, et al. Surfactant Proteins SP-A and SP-D Ameliorate Pneumonia Severity and Intestinal Injury in a Murine Model of Staphylococcus Aureus Pneumonia. Shock. 2016;46:164–72. doi:10.1097/shk.0000000000000587.
21. Aston SJ. Pneumonia in the developing world: Characteristic features and approach to management. Respirology. 2017;22:1276–87. doi:10.1111/resp.13112.
22. Gamberini L, et al. Factors influencing liberation from mechanical ventilation in coronavirus disease 2019: multicenter observational study in fifteen Italian ICUs. J Intensive Care. 2020;8:80. doi:10.1186/s40560-020-00499-4.
23. Mandell LA, et al. Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis. 2007;44(Suppl 2):27–72. doi:10.1086/511159.
24. Feng F, et al. Association of Radiologic Findings with Mortality in Patients with Avian Influenza H7N9 Pneumonia. PLoS ONE. 2014;9:e93885. doi:10.1371/journal.pone.0093885.
25. Hunninghake GW, Gadek J, Kawanami O, Ferrans V, Crystal R. Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. Am J Pathol. 1979;97:149.
26. Baldwin DR, Wise R, Andrews JM, Honeybourne D. Microlavage: a technique for determining the volume of epithelial lining fluid. Thorax. 1991;46:658–62. doi:10.1136/thx.46.9.658.
27. Nayak DK, Mendez O, Bowen S, Mohanakumar T. Isolation and In Vitro Culture of Murine and Human Alveolar Macrophages. J Vis Exp. 2018. doi:10.3791/57287.
28. Yu H, et al. Early prediction of extubation failure in patients with severe pneumonia: a retrospective cohort study. Biosci Rep. 2020;40:BSR20192435. doi:10.1042/BSR20192435.

29. Mughal M, et al. CAN INFLAMMATORY MARKERS PREDICT THE SUCCESSFUL EXTUBATION IN PATIENTS WITH COVID-19? *Chest* **158**, A600-A600, doi:10.1016/j.chest.2020.08.565 (2020).
30. Yao Y, et al. D-dimer as a biomarker for disease severity and mortality in COVID-19 patients: a case control study. J Intensive Care. 2020;8:49. doi:10.1186/s40560-020-00466-z.
31. Güneysel O, Pirmit S, Karakurt S. Plasma d-dimer levels increase with the severity of community acquired pneumonia. Tuberk Toraks. 2004;52:341–7.
32. Huffnagle GB, Dickson RP. The bacterial microbiota in inflammatory lung diseases. Clin Immunol. 2015;159:177–82. doi:10.1016/j.clim.2015.05.022.
33. Chaudhry R, Bordoni B. *StatPearls* (StatPearls Publishing Copyright © 2021. StatPearls Publishing LLC.; 2021.
34. Zaccard CR, Schell RF, Spiegel CA. Efficacy of bilateral bronchoalveolar lavage for diagnosis of ventilator-associated pneumonia. J Clin Microbiol. 2009;47:2918–24. doi:10.1128/jcm.00747-09.
35. Forgiarini SGI, et al. Evaluation of systemic inflammation in patients being weaned from mechanical ventilation. Clin (Sao Paulo). 2018;73:e256–6. doi:10.6061/clinics/2018/e256.
36. Oh DS, Oh JE, Jung HE, Lee HK. Transient Depletion of CD169 + Cells Contributes to Impaired Early Protection and Effector CD8 + T Cell Recruitment against Mucosal Respiratory Syncytial Virus Infection. Frontiers in Immunology 8 (2017).
37. Curtis JL. Cell-mediated adaptive immune defense of the lungs. Proc Am Thorac Soc. 2005;2:412–6. doi:10.1513/pats.200507-070JS.
38. Kolls JK. CD4(+) T-cell subsets and host defense in the lung. Immunol Rev. 2013;252:156–63. doi:10.1111/imr.12030.
39. Norton DL, et al. Bronchoalveolar Tregs are associated with duration of mechanical ventilation in acute respiratory distress syndrome. J Transl Med. 2020;18:427–7. doi:10.1186/s12967-020-02595-3.
40. Myrianthefs P, et al. Mechanical ventilation and the immune response. Intensive Care Med. 2002;28:1365–6. doi:10.1007/s00134-002-1425-0.

Figures

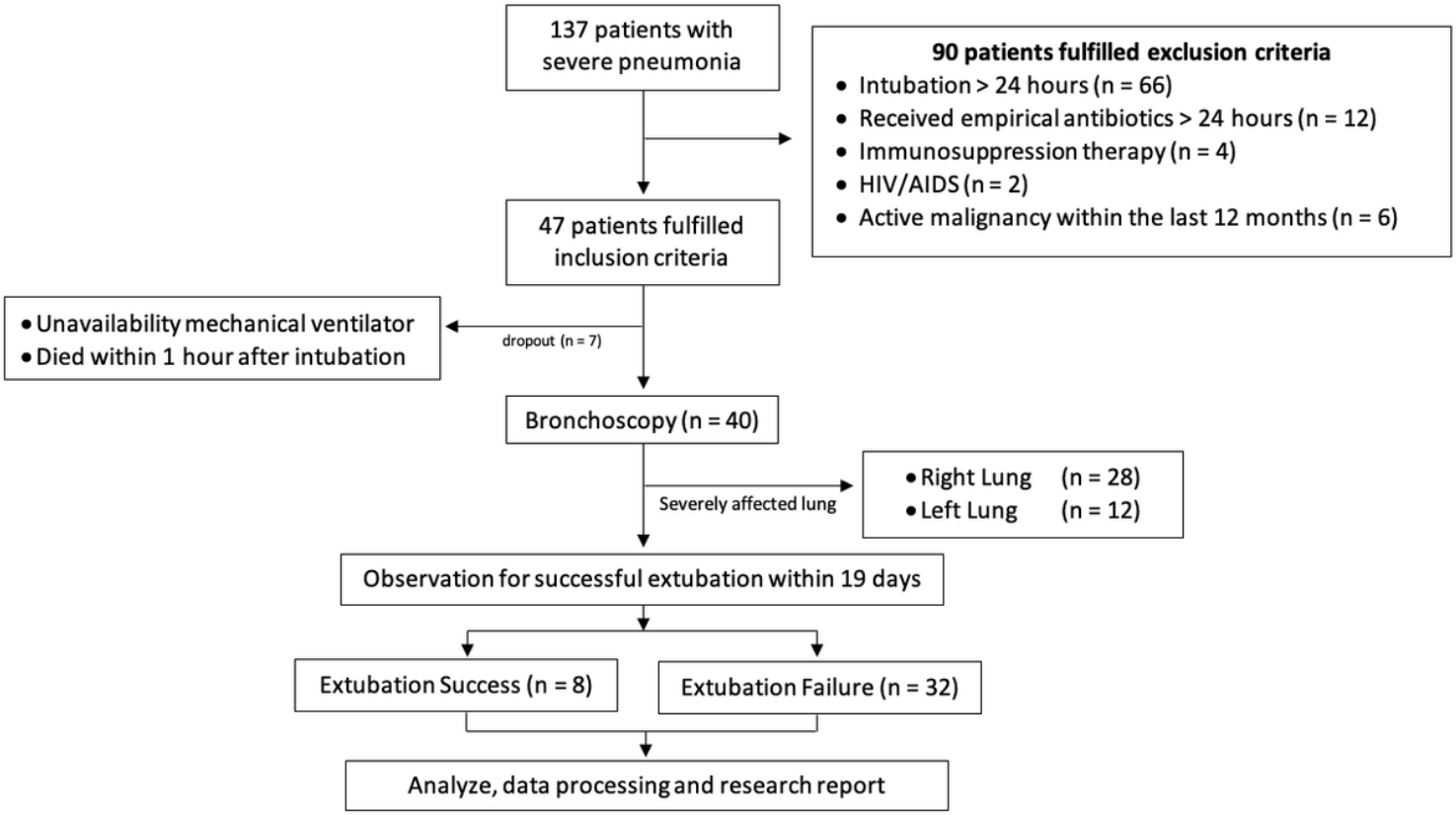

Figure 1

Design and Flow of Participants Through the Study

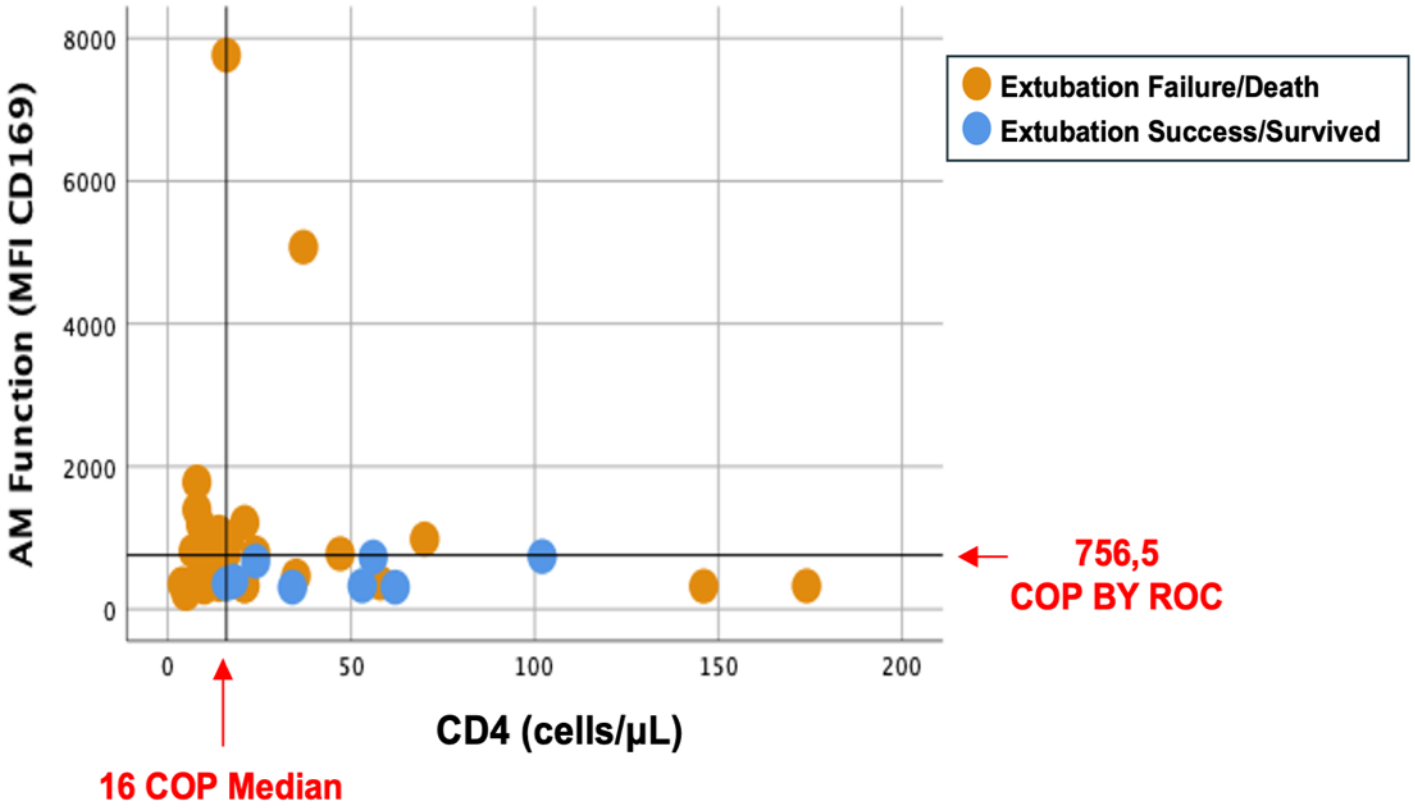

Figure 2

Biomarker Interaction Alveolar Macrophage Function and CD4

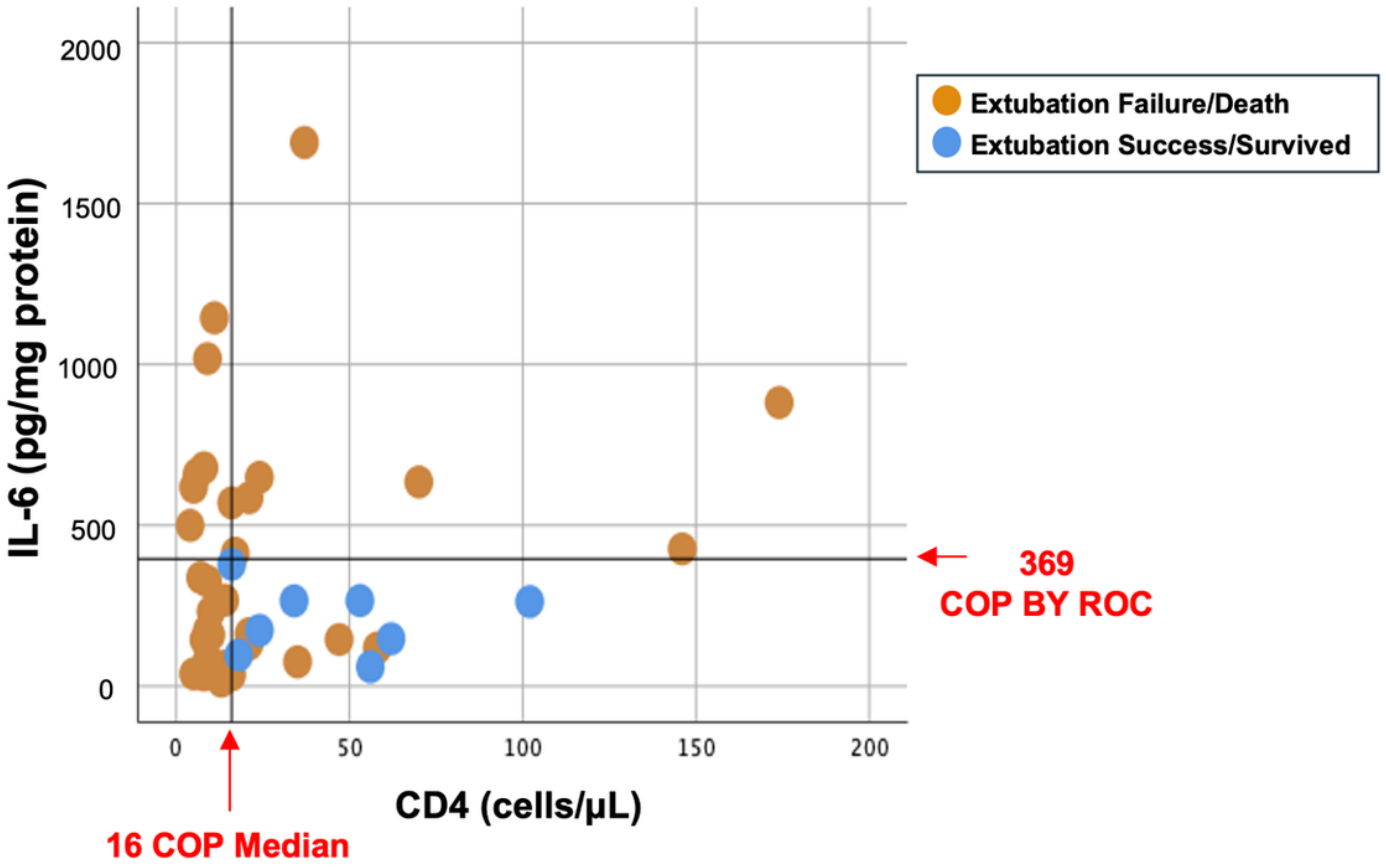

Figure 3

Biomarker Interaction IL-6 and CD4

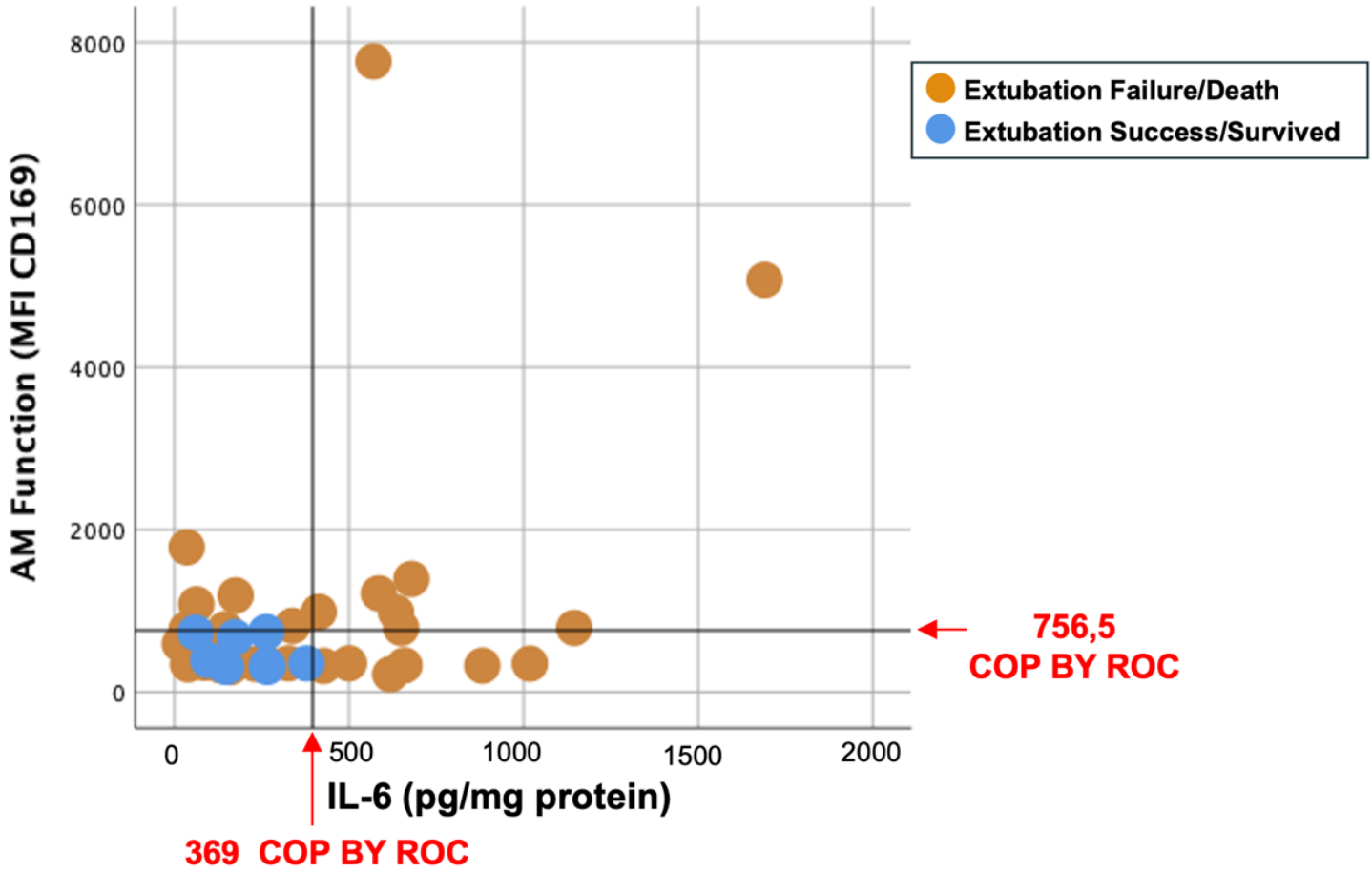

Figure 4

Biomarker Interaction Alveolar Macrophage Function and IL-6

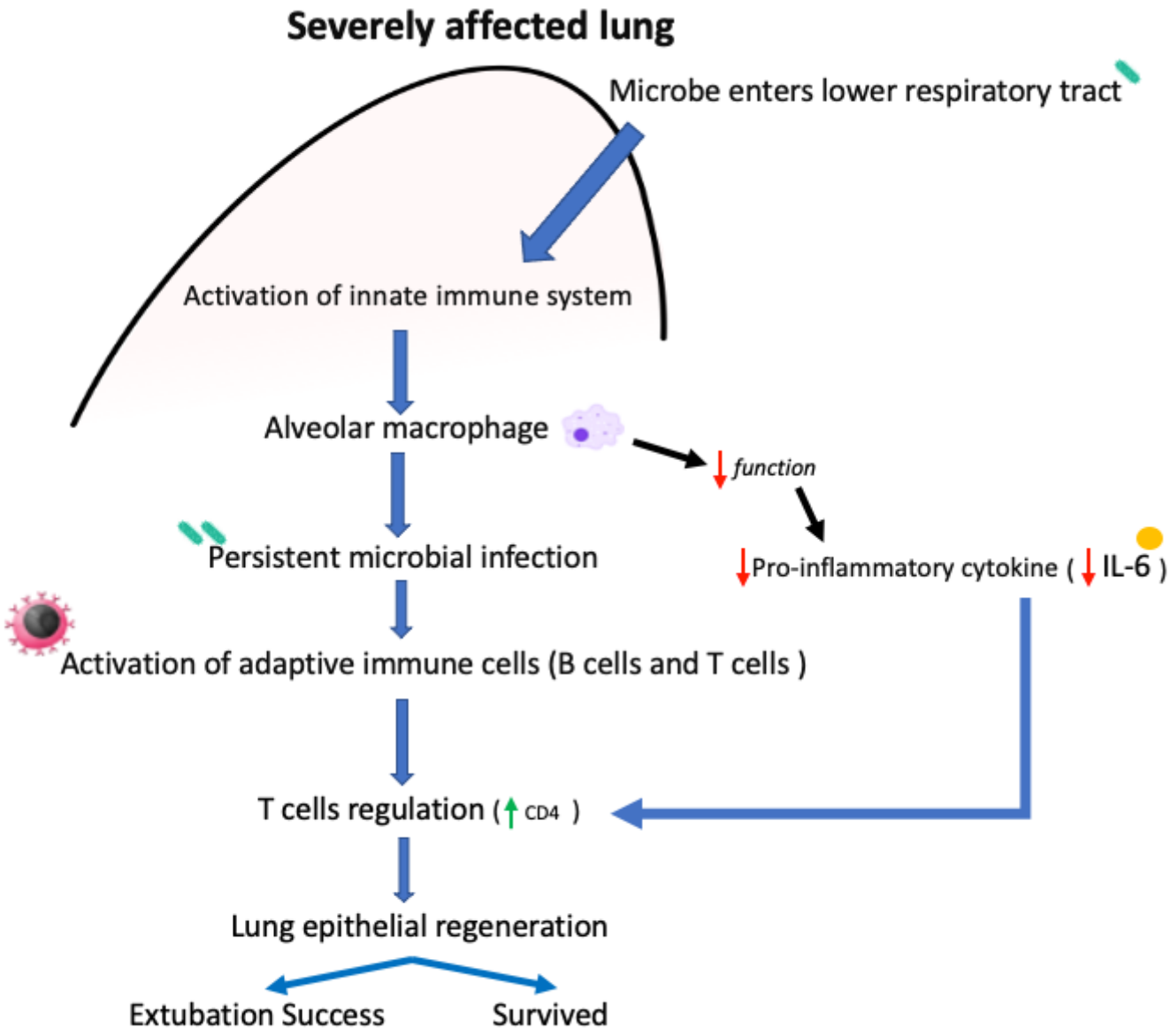

Figure 5

Local Immunopathology Response in Severely Affected Lung of Extubation Success and Survived Severe Pneumonia Patients

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