ORIGINAL ARTICLE

LOW ALVEOLAR MACROPHAGES FUNCTION, LOW BALF IL-6 LEVEL AND HIGH BALF CD4 CELL COUNT IS ASSOCIATED WITH SUCCESSFUL EXTUBATION AND SURVIVAL IN SEVERE PNEUMONIA PATIENTS

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ABSTRACT

Background: To assess Bronchoalveolar-Lavage Fluid (BALF) biomarker inter-relationship of severely affected lung and their association in determining successful extubation and survival.
Methods: This prospective cohort study enrolled one hundred thirty-seven hospitalized severe pneumonia patients admitted to the Resuscitation Emergency Unit/Intensive Care Unit (REU/ICU) of Cipto Mangunkusumo Hospital, Indonesia from November 2020 – January 2021. 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. Univariate analysis was performed for subject characteristics and bivariate analysis was performed for BALF biomarkers (sTREM, alveolar macrophages, IL-6, IL-17, CD4, Treg Foxp3+, SP-A and caspase-3). All p values <.05 were considered statistically significant.
Intervention: Patients who fulfilled inclusion criteria will undergo early bronchoscopy. The BALF was collected from the right and left lungs.
Primary and secondary outcome measures: The primary outcome was the 19-days successful extubation and survival.
Results: 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 = .003) in the left lung and higher absolute CD4+ BALF cell counts (95% Confidence Interval = 9.00 – 29.75, p = .010) in patients with successful extubation and survival. Among all the patients with successful extubation within 19 days, eight patients (100%) displayed the tendency of high CD4 levels (median 16 cells/µL), low expression of alveolar macrophages function (ROC 756.5 MFI CD169), and low expression of IL-6 (ROC 369 pg/mg protein).
Conclusion: BALF low alveolar macrophages function, low IL-6 levels, and high CD4 levels of severely affected lungs is associated with successful extubation and survival in severe pneumonia patients.
Trial registration: The study is registered at UMIN Clinical Trials Registry (UMIN-CTR) (registration number UMIN000046236) on 30/11/2021, accessible at: https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000049197

Keywords: Successful extubation, alveolar macrophages, IL-6, CD4, severe pneumonia.

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BACKGROUND

Pneumonia is the second leading reason for hospitalization 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] 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 macrophages, sTREM-1, mononuclear cells, cytokines, Caspase-3, and SP-A. Each of these biomarkers (sTREM-1[5], alveolar macrophages[6, 7], IL-6[8], IL-17[9], CD4[10, 11], Tregs[12], SP-A[13], or Caspase-3[14]) have been studied independently to mediate local inflammatory responses in severe pneumonia. It has been reported that BALF sTREM-1,[5] alveolar macrophages,[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. We assumed that the inter-relationship of these mediators would orchestrate the immune response outcomes and clinicians will benefit more to understand this complete pathophysiology in severe pneumonia patients.[21] It is unclear how some patients can tolerate the infection and result in successful extubation, whereas others are likely to be in critical condition that leads to extubation failure and/or mortality. Previous studies have not comprehensively explained the biomarker inter-relationship of severely and less severely affected lungs.

METHODS

A total of 137 hospitalized severe pneumonia patients were admitted to the Resuscitation Emergency Unit/Intensive Care Unit (REU/ICU) ward of Cipto Mangunkusumo Hospital (a National Referral Hospital) from November 2020 – January 2021. Patients who fulfilled the inclusion criteria underwent bronchoscopy. Demographic and clinical characteristics, laboratory findings, and eight BALF biomarkers (alveolar macrophages amount and function, sTREM-1, IL-6, IL-17, CD4, Tregs, Caspase-3, and SP-A) were recorded. Informed consent for bronchoscopy and participation in the study was obtained from all subjects and/or their legal guardian(s).

Definitions

The 19-day successful extubation was selected based on the[22] maximum mechanical ventilation duration reported by Gamberini *et al.*[23] Extubation failure was defined as patients being reintubated in 48 hours, re-intubation, and/or reused ventilator after successful extubation, and/or death.[24] Early extubation was defined as successful endotracheal tube removal in no less than 24 hours field[25] without the need to reintubate and reuse the mechanical ventilator afterward.

Study Design

This is a prospective cohort study and patients were recruited by consecutive sampling in REU/ICU ward, Cipto Mangunkusumo National Hospital. 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. Dropout criteria: unavailability of the mechanical ventilator; died within 12 hours after intubation.

Patient and Public Involvement

Patients and Public were not involved in the design, conduct, reporting, and dissemination plans of our research. The study result will be disseminated through publication.

BALF Collection

Bronchoscopy was performed an average of 4 hours after patients' intubation. The anesthesiologist gave intravenous midazolam and propofol to give optimum sedation. Chest radiography was performed to determine the severely and less severely affected lung, as discussed by the two Respirologists and

Critical Illness consultants, and one internist, based on chest imaging severity score proposed by Feng *et al.*[26]

The order of BAL suctioning was initially performed from the less severely affected lung (subsegment of the right middle lobe and lingula of the left lung) and proceeded to the severely affected lung. Severely and less severely affected lung BALF were analyzed separately. Bronchoalveolar lavage was performed (standard guidance field[27]) by serial 20 mL fractions of 0.9% of normal saline solution to a total volume of 100 mL (room temperature). A 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.

BALF Preparation

The BALF specimen containers were inserted in a sterilized medical plastic bag before being transferred 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. BALF specimens were collected in a 50 mL tube and then centrifuged at 1000 g for 10 min. BALF supernatant was separated to analyze sTREM-1, IL-6, IL-17, and SP-A and frozen at -80 ^{o}C. BALF pellet was suspended in 2 mL PBS to analyze alveolar macrophages, Tregs, and Caspase-3. For Caspase-3, the sample was frozen at -80^{o}C before transfer.

BALF Analysis

Flow cytometry was used to analyze alveolar macrophages, 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.[28] 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 macrophages 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 macrophages 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^{TM} 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^{TM} II flow cytometer (BD Biosciences, New Jersey, USA). BD FASCDiva^{TM} software version 6.1.3 (BD Biosciences, New Jersey, USA) was used to perform the cytometric analysis.

The amount and function of alveolar macrophages were analyzed.[29] Alveolar macrophages (amount) was analyzed according to the percentage of macrophage cells / CD45^{+}, HLA-DR^{+}, and CD11b^{+}. Alveolar macrophages (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 25^{th} – 75^{th} percentile), and percentages. Data normality was assessed based on the 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 and bivariate analysis was performed for BALF biomarkers (sTREM, alveolar macrophages, IL-6, IL-17, CD4, Treg Foxp3+, SP-A and caspase-3). All *p values* <.05 were considered statistically significant.

Statistically significant BALF biomarker(s) will be correlated to other BALF biomarkers in patients with successful extubation and survived. Method of cutoff points obtained using median or area under curve method. Cutoff points by ROC are obtained by calculating the 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

A total of 137 severe pneumonia patients were recruited within November 2020 through January 2021. Forty patients underwent bronchoscopy for BALF collection. The right lung was the predominant severely affected lung. Eight patients were successfully extubated and survived (Figure 1).

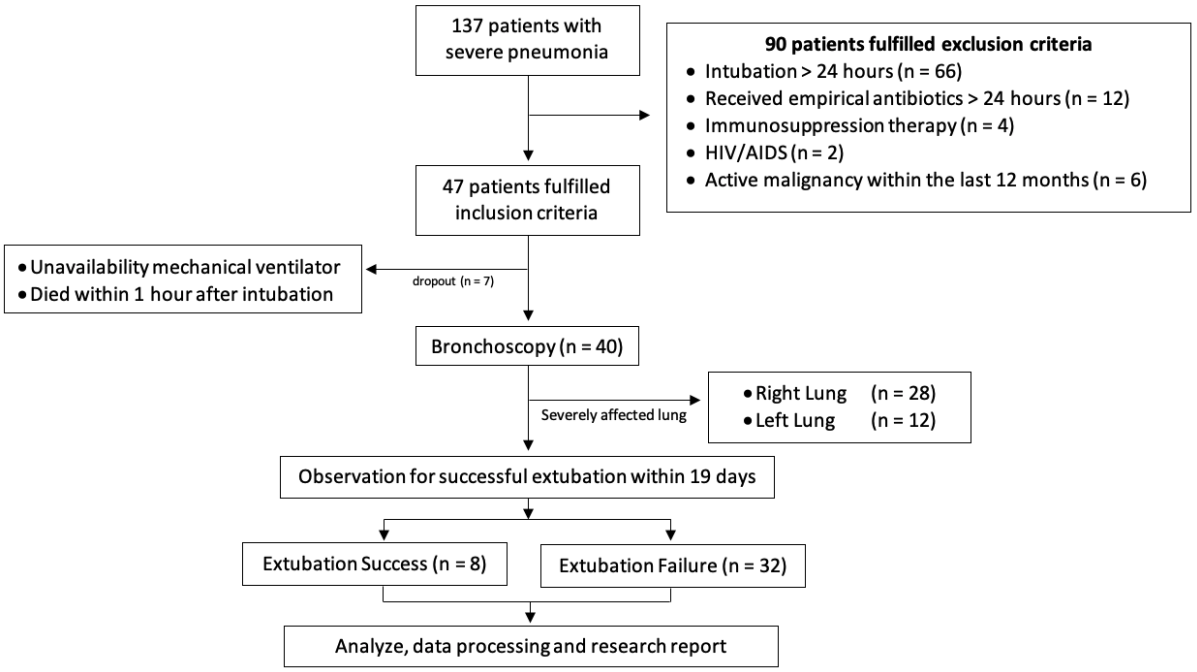

Figure 1. Design and Flow of Participants Through the Study.

In the group of patients with successful extubation and survived (8 patients), there were 4 patients of male (50%), with a 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 ($p < .001$) in the patients with successful extubation and survived (Table 1).

Table 1. Demographic and Clinical Characteristics of the Study Population

Characteristic	All Patients (n = 40)	Successful Extubation & Survival (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)	.576
BMI (kg/m^2), (±SD)	26.7 (±3.2)	27.9 (±3.6)	26.4 (±3.2)	.262
Smoker, n (%)	8 (20)	0 (0)	8 (25)	.173
Severely affected lung, (Right Lung, %)	28 (70)	4 (50)	24 (75)	.211
Length of hospital stays (days), median (IQR)	8 (1-80)	21 (15 – 26.5)	6.5 (4 – 10.5)	**< .001**
Comorbidities				
Diabetes Mellitus, n (%)	22 (55)	5 (62.5)	17 (53)	.709
Hypertension, n (%)	20 (50)	5 (62.5)	15 (47)	.696
Chronic Kidney Disease, n (%)	17 (42.5)	2 (25)	15 (47)	.428
COPD, n (%)	2 (5)	0 (0)	2 (6.25)	1
Bronchial Asthma, n (%)	2 (5)	1 (12.5)	1 (3.12)	.364
Cardiovascular Disease, n (%)	2 (5)	1 (12.5)	1 (3.12)	.364
Systemic Lupus Erythematosus, n (%)	1 (2.5)	0 (0)	1 (3.12)	1
Obesity, n (%)	1 (2.5)	1 (12.5)	0 (0)	.200
Scoring System				
APACHE II	16 (12.5 – 21)	14.63 (±3.42)	18.19 (±6.23)	.129
mSOFA	9 (8 – 11)	8 (8 – 9)	9 (8 – 12)	.169

p value < .05 (statistically significant).
BMI: Body Mass Index, COPD: Chronic Obstructive Pulmonary Disease, APACHE: Acute Physiology and Chronic Health Evaluation, mSOFA: Modified Sequential Organ Failure Assessment.

There was a significantly higher D-dimer value (*p* = .010) in the patients with successful extubation and survived (Appendix A). There were significantly higher absolute CD4+ BALF
cell counts (95% Confidence Interval = 9.24 – 49.50, *p* = .003) in the left lung of severe pneumonia patients (Appendix B). Other BALF biomarkers (Appendix C). There were significantly higher absolute CD4+ BALF cell counts (95% Confidence Interval = 9.00 – 29.75, *p* = .010) in the patients with successful extubation and survived (Table 2). Other BALF biomarkers (Appendix D). Microbial patterns of the eight patients who were successfully extubated and survived (Appendix E).

Table 2. BALF Findings in Severely Affected Lung Based on Extubation Status

BALF Findings	Total (n = 40)	Successful Extubation & Survival (n = 8)	Extubation Failure & Death (n = 32)	*p* Value
Alveolar Macrophages (MFI CD169)	396 (334.50 – 802)	373 (317.50 – 701.00)	432.50 (342.50 – 984.00)	.187
Absolute CD4+ (cells/µL)	16 (9 – 36)	43.50 (21.00 – 59.00)	13.50 (8.50 – 22.50)	**.010**
IL-6 (pg/mg protein)	264.21 (124.77 – 577.570)	218.25 (121.85 – 265.68)	296.71 (124.77 – 626.45)	.310

p Value < .05 (statistically significant).
BALF: Bronchoalveolar Lavage Fluid, MFI: Mean Fluorescence Intensity, CD: Cluster of Differentiation, IL: Interleukin.

Among all the patients with successful extubation within 19 days, 8 patients (100%) displayed the tendency of high CD4 levels (cutoff points median 16 cells/µL), low expression of alveolar macrophages function (cutoff points by ROC 756.5 MFI CD169), 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), and the tendency of low IL-6 levels (cutoff points by ROC 396 pg/mg protein) with low expression of alveolar macrophages function (cutoff points by ROC 756.5 MFI CD169) (Table 3). Other BALF biomarker inter-relationship (Appendix F)

Table 3. BALF Inter-relationship of Alveolar Macrophages, IL-6, and CD4

Extubation status	Yes	No	Total	PR*	*p* Value

	n	%	n	%	n	%	(95% CI)	
a. High CD4 and Low Functional Alveolar Macrophages								
Extubation failure	26	100	6	43	32	80	0.18 (0.09–0.30)	< .001
Successful Extubation	0	0	8	57	8	20		
Total	26	100	14	100	40	100		
b. High CD4 and IL-6								
Extubation failure	26	100	6	43	32	80	0.18 (0.09–0.30)	< .001
Successful Extubation	0	0	8	57	8	20		
Total	26	100	14	100	40	100		
c. Low IL-6 and Low Functional Alveolar Macrophages								
Extubation failure	20	100	12	60	32	80	0.37 (0.24–0.58)	.002
Successful Extubation	0	0	8	40	8	20		
Total	20	100	20	100	40	100		

p Value < .05 (statistically significant).
*PR: Prevalence *Ratio*, CI: Confidence Interval, IL: Interleukin, CD: Cluster of Differentiation.

a. Extubation less than 20 days and survived, 8 subjects (100%) displayed the trend of high CD4 level (Cut-off Point median 16 cell/µL) with low expression of functional alveolar macrophages (Cut-off Point by ROC 756,5 MFI CD169) **(PR = 0.18; 95% CI= 0.09-0.3; ρ< .001)**. **b.** Extubation less than 20 days and survived, 8 subjects (100%) displayed the trend of high CD4 level (Cut-off Point median 16 cell/µL) with low expression of IL-6 (Cut-off Point by ROC 396) **(PR = 0.18; 95% CI= 0.09-0.3; ρ< .001)**. **c.** Extubation less than 20 days and survived, 8 subjects (100%) displayed the trend of low IL-6 level (Cut-off Point by ROC 396) with low expression of functional alveolar macrophages (Cut-off Point by ROC 756,5 MFI CD169) **(PR = 0.37; 95% CI=0.24-0.58; ρ = .002).**

DISCUSSION

Based on the sample size calculation, 1010 patients were required. Due to the pandemic

COVID-19 in our country, after the enrollment of 40 patients, an internal discussion was held, and the team members decided not to continue the patients' enrollment.

Previous studies have described several predictors to determine extubation failure.[30, 31] Our findings reported that the demographic and lab profile were not that effective in determining extubation failure. Further analysis of immune biomarkers is required to evaluate the successful extubation and survival in severe pneumonia. This study found that absolute CD4+ BALF cell counts were significantly higher in the patients with successful extubation and survived (43.50 cells/µL vs 13.50 cells/µL, p = .001). By analyzing BALF biomarkers inter-relationship (alveolar macrophages amount and function, sTREM-1, IL-6, IL-17, CD4, Tregs, caspase-3, and SP-A), we found BALF biomarker inter-relationship in patients with successful extubation and survived (8 patients) marked by low alveolar macrophages function, low IL-6 levels, and high CD4 levels.

Patients with successful extubation/survived had longer hospital stays, affecting cost-effectiveness and nosocomial infection risks. Based on the laboratory findings, there were no significant differences, except for the D-dimer values. 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,[32, 33] 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.[34, 35] A study of bronchoalveolar lavage efficacy reported that unilateral sampling of the right lung had 89% efficacy compared to bilateral sampling in both lungs (81.5%).[36] 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.[34]

Previous studies have investigated the role of systemic inflammatory biomarkers to predict the extubation status in severe pneumonia patients.[37] However, to our knowledge, no previous studies had evaluated the local inflammatory biomarkers, particularly in the right and left severely affected lungs. Previous studies showed that sTREM-1,[5] alveolar macrophages,[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 macrophages[6, 7], IL-6[8], IL-17[9], CD4[10, 11], Tregs[12], SP-A[13], or Caspase-3[14]) have been studied independently to mediate local inflammatory responses in severe pneumonia patients.

Our study showed that severe pneumonia patients with successful extubation and survived tended to have low alveolar macrophages function with low IL-6 levels. Alveolar macrophages function and IL-6 are considered the mediators/markers of local innate immunity, whereas CD4 is a mediator/marker for adaptive immunity.[38, 39] In previous reports, the role of alveolar macrophages function (marked by expression of CD169+) has been described to enhance pro-inflammatory responses, including the secretion of IL-6.[38] Patients with successful extubation and survived showed that the severely affected lung had high CD4 levels, which indicates that the increased response 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.[40] The role of BALF CD4+ T-cells have 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 successful extubation.[41] Our study also reported the levels of inflammatory biomarkers for Tregs [Foxp3+ CD25+ / CD4 (%)]. 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 successful extubation and survival of the patients. Our study showed that the right lung was 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 the depletion of CD4+ T cells is associated with the extubation failure and death.

This study demonstrates 19 days of successful extubation and survival in severe pneumonia patients are associated with BALF biomarker inter-relationship of low alveolar macrophages function, low IL-6 levels, and high CD4 levels BALF (severely affected lung), (Figure 2). These findings should be specially analyzed in severe pneumonia patients to evaluate successful extubation.

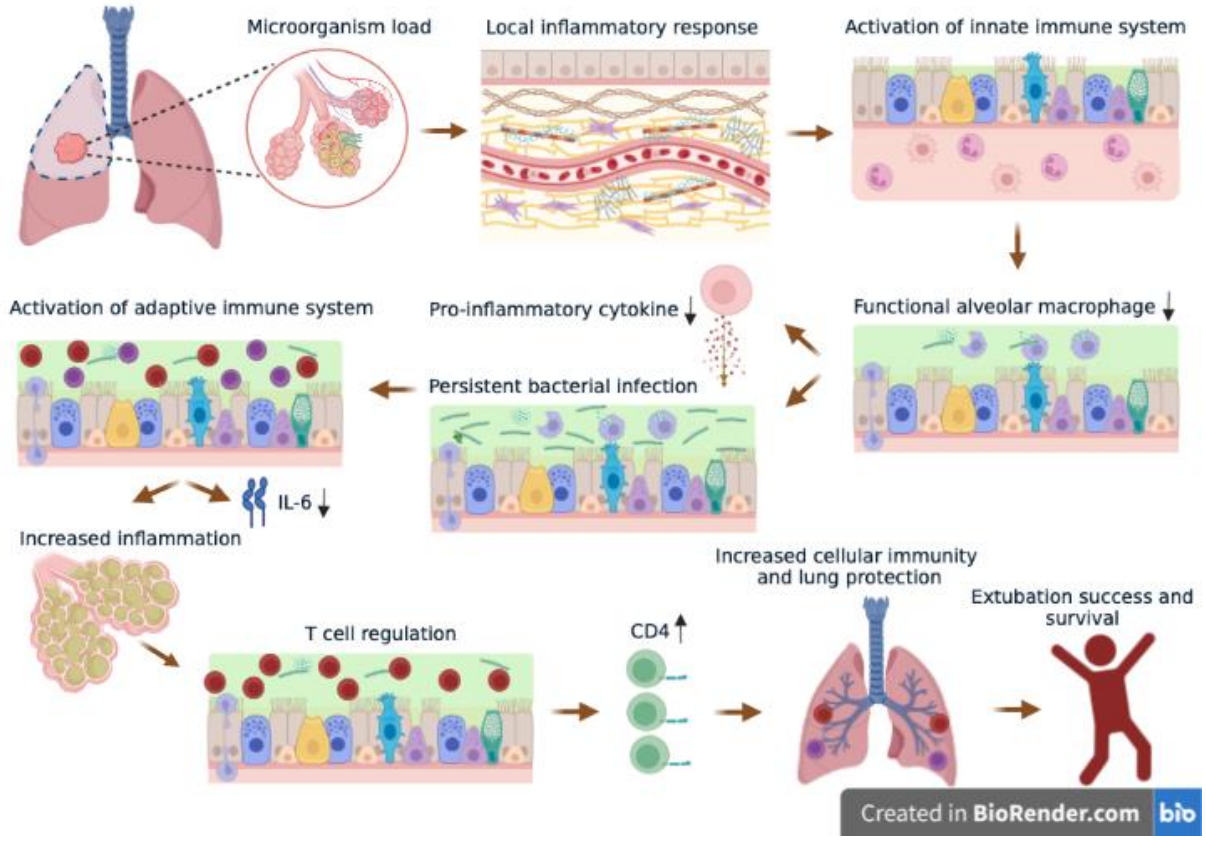

Figure 2. Local Immunopathology Response in Severely Affected Lung of Successful Extubation and Survival in Severe Pneumonia Patients.
Invasion of microorganisms into lower respiratory tract will trigger local inflammatory response marked by the activation of innate immune system. Low functional alveolar macrophages will reduce the secretion of IL-6 pro-inflammatory cytokine. Persistent bacterial infection will activate the adaptive immune system (B cell and T cell) that will regulate T cell function. High level of CD4 cell count will enhance cellular immunity and lung protection against tissue damage in severe pneumonia patients with successful extubation and survival

Our study had several important limitations. First, this was a single-center study with a small sample size and this is due to the COVID-19 pandemic situation in our country that did not allow us to conduct a multicenter study with the actual planned sample size. Second, no comparison of local and systemic inflammation markers was analyzed. The strength of this study includes the novel finding of BALF biomarker inter-relationship, which might help to develop a new pathophysiology concept in severe pneumonia. To minimize mechanical ventilation's intervention risks in altering immune responses,[42] we performed bronchoscopy an average of four hours after patients' intubation. Although all patients with successful extubation and survival showed the BALF biomarker inter-relationship (alveolar macrophages function, IL-6, and CD4), future multicenter studies are required to evaluate serial BALF biomarkers inter-relationship in severe pneumonia to predict successful extubation.

In conclusion, severely affected lungs of severe pneumonia patients with successful extubation and survival, we found BALF biomarker inter-relationship marked by low alveolar macrophage function, low IL-6 levels, and high CD4 cell count levels. This BALF inter-relationship can be an option for clinicians to assess successful extubation and survival in severe pneumonia patients.

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DECLARATIONS

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Human/Animal Ethics Approval Declaration: 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/hospital review committee, and the informed consent was obtained from all subjects and/or their legal guardian(s). All the methods included in the study are in accordance with the declaration of Helsinki.

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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