

Low BALF CD4 T cells count is associated with extubation failure and mortality in critically ill covid-19 pneumonia

Gurmeet Singh^a, Cleopas Martin Rumende^a, Surendra K. Sharma^{b,c}, Iris Rengganis^d, Zulkifli Amin^a, Tonny Loho^e, Emmy Hermiyanti^f, Kuntjoro Harimurti^g and Heri Wibowo^h

^aDepartment of Internal Medicine, Faculty of Medicine, Division of Respiriology and Critical Illness, Universitas Indonesia – Cipto Mangunkusumo General Hospital, Jakarta, Indonesia; ^bDepartment of Molecular Medicine, Jamia Hamdard Institute of Molecular Medicine, Hamdard University, New Delhi, India; ^cDepartment of General Medicine & Pulmonary Medicine, JNMC, Datta Meghe Institute of Medical Science, New Delhi, India; ^dDepartment of Internal Medicine, Faculty of Medicine, Division of Allergy and Clinical Immunology, Universitas Indonesia, Cipto Mangunkusumo General Hospital, Jakarta, Indonesia; ^eDepartment of Clinical Pathology, Faculty of Medicine, Universitas Indonesia – Cipto Mangunkusumo General Hospital, Jakarta, Indonesia; ^fDepartment of Internal Medicine, Faculty of Medicine, Division of Respiriology and Critical Illness, Universitas Padjadjaran, Dr Hasan Sadikin Hospital Bandung, Bandung, Indonesia; ^gDepartment of Internal Medicine, Faculty of Medicine, Division of Geriatrics, Universitas Indonesia – Cipto Mangunkusumo General Hospital, Jakarta, Indonesia; ^hHead of Integrated Laboratory, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

ABSTRACT

Background: Critically ill COVID-19 pneumonia is one of the main causes of extubation failure and mortality. Understanding clinical characteristics, laboratory profiles and bronchoalveolar lavage fluid (BALF) immunopathology may help improve outcomes in critically ill COVID-19 pneumonia. We aimed to describe clinical characteristics, laboratory profiles and BALF immunopathology based on lung severity in critically ill COVID-19 pneumonia patients.

Materials and methods: Forty critically ill severe pneumonia patients requiring invasive mechanical ventilation in Cipto Mangunkusumo General (National Tertiary Referral Hospital), Indonesia within November 2020–January 2021 were enrolled in this study. Early BALF collection was performed after patients' intubation. Clinical characteristics, laboratory profiles and BALF biomarkers (sTREM-1, alveolar macrophage amount and function, IL-6, IL-17, CD4 T-cells, Tregs, SP-A and Caspase-3) were observed and analysed. Outcomes were measured based on extubation failure (within 19 days) and 28-days mortality. Univariate and bivariate analyses were performed.

Results: Early bronchoscopy was performed in an average of 4 h (SD = 0.82) after patients' intubation. Twenty-three and twenty-two patients had extubation failure (within 19 days) and 28-days mortality, respectively. In the baseline clinical characteristics of critically ill COVID-19 patients, we found no significant differences in the extubation and mortality status groups. In the laboratory profiles of critically ill COVID-19 patients, we found no significant differences in the extubation status groups. In critically ill COVID-19 pneumonia patients, there was a significant high D-dimer levels in survived group ($p = .027$), a significant low BALF CD4 T-cells count in the right lung ($p = .001$) and a significant low BALF CD4 T-cells count ($p = .010$ and $p = .018$) in severely affected lung with extubation failure and mortality.

Conclusions: BALF CD4 T-cells count evaluation of severely affected lung is associated with early extubation failure and mortality in critically ill COVID-19 pneumonia patients.

KEY MESSAGE

- Few studies have been conducted during the peak COVID-19 period analysing combined bronchoalveolar lavage fluid (BALF) immunopathology biomarkers within four hours of intubation to assess extubation failure and mortality. In this study, we reported eight BALF immunopathology biomarkers (sTREM-1, alveolar macrophage, IL-6, IL-17, CD4 T-cells, Tregs, SP-A and Caspase-3).
- We found significantly low BALF CD4 T-cells count in the right lung, and low BALF CD4 T-cells count in severely affected lung of critically ill COVID-19 pneumonia patients in extubation failure and mortality.

Abbreviations: ACE: angiotensin-converting enzyme; APACHE: acute physiology and chronic health evaluation; BAL: bronchoalveolar lavage; BALF: bronchoalveolar lavage fluid; CCI: Charlson Comorbidity Index; CD: cluster of differentiation; COVID-19: coronavirus disease 2019; CRP: C-

ARTICLE HISTORY

Received 11 February 2022
Revised 18 June 2022
Accepted 21 June 2022

KEYWORDS

Critically ill COVID-19 pneumonia; BALF CD4 T-cells count; extubation failure

reactive protein; ELISA: enzyme-linked immunoassay; ESR: erythrocyte sedimentation rate; ICU: intensive care unit; IFN: interferon; IL: interleukin; IQR: interquartile range; mSOFA: modified sequential organ failure assessment; PaO₂/FiO₂: the ratio of arterial oxygen partial pressure to fractional inspired oxygen; PBS: phosphate buffer saline; REU: resuscitation emergency unit; RT-PCR: real-time polymerase chain reaction; SARS: severe acute respiratory syndrome; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; SBT: spontaneous breathing trial; SD: standard deviation; SLE: systemic lupus erythematosus; SP-A: surfactant protein-A; sTREM-1: soluble triggering receptor expressed on myeloid cells-1; Tregs: regulatory T cells; TNF: tumour necrosis factor

Introduction

Pneumonia involves persistent local inflammatory reactions which potentially increase disease severity due to the inability to control microbial invasion. Comprehensive knowledge regarding local inflammation may help understand the local host response to microorganisms [1]. The number of viral pneumonia cases increased during the coronavirus disease 2019 (COVID-19) pandemic [2]. Alteration of immune response (loss of immune control) in COVID-19 can prevent viral elimination and recovery [3]. A significant number of patients with critically ill COVID-19 pneumonia experienced severe respiratory failure, necessitating admission to the intensive care unit (ICU), with almost 80% of them requiring mechanical ventilation [4]. Due to various organ involvement and dynamic development over time, these patients exhibit high complexity. Various studies have described comorbidities, clinical scoring systems and laboratory profiles to determine the disease severity, ICU admission, the need for mechanical ventilation, and mortality [4–10]. However, little information is known concerning local immunopathology responses with the increased usage of the mechanical ventilator. Understanding clinical characteristics and bronchoalveolar lavage fluid (BALF) immunopathology in the severely and less severely affected lung may help to improve outcomes in critically ill COVID-19 pneumonia patients [11]. Additionally, the study of local inflammatory biomarkers of the lung is required to measure the severity of lung injury and determine the risk for extubation failure and mortality [12]. Previous studies have been conducted to analyse local immunopathology responses in sputum and bronchoalveolar lavage (BAL) fluid observing inflammatory profiles (i.e. cytokine interleukin (IL)-6, IL-8, IL-10, IL-17, IL-1 β , IL-1ra, tumour necrosis factor- α (TNF- α) and interferon- γ (IFN- γ) [1,13], moreover, no study has been done to evaluate BALF immunopathology biomarker (sTREM-1, alveolar macrophage, IL-6, IL-17, CD4 T-cells, Tregs, SP-A and Caspase-3) based on the severity in the right and left lung of critically ill COVID-19 pneumonia patients. Each of these

immunopathology biomarkers (sTREM-1 [14–18], alveolar macrophage [19–22], IL-6 [23–25], IL-17 [26–29], CD4 T-cells [30–32], Tregs [33–35], SP-A [36,37] or Caspase-3 [38,39]) has been studied independently to mediate local inflammatory responses in severe pneumonia patients. Based on human and animal BALF studies, we assumed that BALF sTREM-1 [18], alveolar macrophage [20], IL-6 [13], IL-17 [28], CD4 T-cells and Tregs [35], SP-A [40], and Caspase-3 [37] played a crucial role in local immunopathology (innate and adaptive immune responses) in critically ill COVID-19 pneumonia patients. Therefore, this study may give a better knowledge to understand wider perspective about BALF immunopathology biomarkers to determine outcomes in critically ill COVID-19 pneumonia patients.

The main aim of this study is to describe BALF immunopathology based on severity in right and left lung of critically ill COVID-19 pneumonia patients on mechanical ventilation hospitalized in Resuscitation Emergency Unit/Intensive Care Unit (REU/ICU). We analysed BALF biomarkers which may be associated with extubation failure (within 19 days) and 28-days mortality.

Materials and methods

Forty critically ill severe pneumonia patients were enrolled in Cipto Mangunkusumo General (National Tertiary Referral Hospital), Indonesia within November 2020 to January 2021. The main teaching hospital for Universitas Indonesia is Cipto Mangunkusumo General Hospital (National Tertiary Referral Hospital). Inclusion criteria: aged 18 years or older; severe pneumonia (based on IDSA/ATS 2007 criteria and COVID-19 WHO guidelines confirmed by positive BALF PCR SARS-CoV-2); can undergo bronchoscopy within 12 h of admission to REU/ICU; receive empirical antibiotics of no more than 24 h; and intubated within 24 h. 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. Clinical characteristics, laboratory profiles and eight BALF biomarkers (sTREM-1, alveolar macrophage (amount and function), IL-6, IL-17, CD4 T-cells, Tregs, SP-A and Caspase-3) were recorded and analysed. This study has been approved by Ethics Committee Universitas Indonesia (approval number: KET-171/UN2.F1/ETIK/PPM.00.02/2020) and Ethics Committee of Health Research, Cipto Mangunkusumo Hospital. Informed consent was approved by ethics committee (approval number: 0995/rev00/KEP/2020). Informed consent for bronchoscopy was signed by patient's family member (patients were intubated).

Study design

The present study was designed as an observational cross-sectional study, in which eligible patients were classified into extubation (success and failure) group and mortality (survived and non-survived) group. Critically ill COVID-19 pneumonia patients will be further analysed to evaluate BALF immunopathology.

Definition of extubation failure and mortality

The 19-days extubation failure was selected based on the study reported by Gamberini et al. (median 20-days extubation failure) [6], and 28-days mortality observation was chosen according to the reported pneumonia studies on mortality prediction [5,9]. Extubation failure was defined as patients being reintubated and/or reusing ventilator after successful extubation in 48 h, and/or death within 19 days [6,41]. Twenty-eight days mortality was defined as patients being recorded died in REU/ICU. Patients' mortality outside REU/ICU (i.e. inward, after a hospital discharged) was not recorded as 28-days mortality.

Assessment of lung severity

Radiographic chest X-ray findings were reported by radiologists prior to BALF collection. Severely and less severely affected lung were discussed by two respirologist and critical illness consultants, plus one internist, based on the affected lung chest imaging severity scoring proposed by Feng et al. [42].

Bronchoalveolar lavage fluid collection

Early BALF collection was performed after patients' intubation. Intravenous midazolam and propofol were given by the anaesthesiologist in order to give

optimum sedation during bronchoscopy. The order of BAL suctioning was initially performed from the less severely affected lung and proceeded to the severely affected lung, from the subsegment of the right middle lobe and lingula of the left lung. Severely and less severely affected lung BALF were analysed separately. Bronchoalveolar lavage was performed (standard guidance [43]) 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 h. Patients were observed for one-hour post-procedure.

Bronchoalveolar lavage fluid preparation

The BALF specimen containers were inserted in 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 a sufficient protective equipment including protective, disposable aprons, N95 masks, goggles and double layer hand gloves. Specimen volume was evaluated for appearance, colour, clearness and contamination with intrabronchial blood. Bronchoalveolar lavage fluid specimens were collected to 50 mL tube and then centrifuged at 1000×g for 10 min. Bronchoalveolar lavage fluid supernatant was separated to analyse sTREM-1, IL-6, IL-17 and SP-A and frozen at –80 °C. Bronchoalveolar lavage fluid pellet was suspended in 2 mL PBS (phosphate buffer saline) to analyse alveolar macrophage, Tregs and Caspase-3. For Caspase-3, sample was frozen at –80 °C prior to transfer to Integrated Laboratory of Medical Faculty, Universitas Indonesia.

Bronchoalveolar lavage fluid analysis

Flow cytometry was used to analyse alveolar macrophage, CD4 T-cells and Tregs. Enzyme-linked immunosorbent assay (ELISA) was used in duplicate to analyse sTREM-1 (MyBioSource ELISA kits, San Diego, CA), IL-6 and IL-17 (R&D systems quantikine ELISA kits, Minneapolis, MN), SP-A (LSBio's ELISA kits, Seattle, WA) and Caspase-3 (Cusabio ELISA kits, Houston, TX). Interleukin-6, IL-17 and SP-A were observed from BALF supernatant. Caspase-3 was observed from cell pellet homogenate and were extracted by using freeze-thawing method (this process was done for 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 [44]. Bradford technique (Bio-RAD, Hercules, CA) 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 min, followed by insertion of FACS lysing solution for 15 min. The working panel of mAbs at five colour assays used for alveolar macrophage and Tregs BALF evaluation was 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, Franklin Lakes, NJ). The resulting cell pellet of CD4 T-cells was incubated at room temperature in the dark, with mAbs in BD TruCount™ Tubes for 15 min, followed by insertion of FACS lysing solution for 15 min at room temperature with following mAbs working panel: CD45+/CD3+/CD4+/CD8+ (BD Biosciences, Franklin Lakes, NJ). Specimens were acquired with a FACSCanto™ II flow cytometer (BD Biosciences, Franklin Lakes, NJ) after been washed. BD FASCDiva™ software version 6.1.3 (BD Biosciences, Franklin Lakes, NJ) was used to perform the cytometric analysis.

Amount and function of alveolar macrophage were analysed according to procedure proposed by Nayak et al. [45] Alveolar macrophage (amount) was analysed according to percentage macrophage cells/CD45⁺, HLA-DR⁺ and CD11b⁺. Alveolar macrophage (function) was analysed according to the mean fluorescence intensity (MFI) of CD169⁺.

Statistical analysis

Numerical and categorical variables were reported through mean, and standard deviation or median, and interquartile range (IQR), and proportions. Data normality was assessed based on Shapiro–Wilk's 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 (sTREM-1, alveolar macrophage, IL-6, IL-17, CD4 T-cells, Tregs, SP-A and

Caspase-3). All *p* values <.05 were considered statistically significant. SPSS Statistic version 26 software (IBM Corporation, Armonk, NY) was used to analyse all recorded data.

Results

Based on our national guidelines, all critically ill COVID-19 pneumonia patients in this study received intravenous dexamethasone, N-acetyl cysteine, low dose vitamin D supplementation, empirical antibiotics; and four patients received tocilizumab. Bronchoalveolar lavage fluid collection was performed in an average of 4 h (SD = 0.82) after patients' intubation. Twenty-three and twenty-two patients had extubation failure and mortality, respectively.

Among 40 severe pneumonia patients, 28 critically ill COVID-19 pneumonia patients were further analysed to evaluate BALF immunopathology. Twelve patients were excluded (non-COVID-19) from this study. The baseline clinical characteristics of critically ill COVID-19 patients based on extubation and mortality status are shown in Tables 1 and 2, respectively. In the extubation status, we found no significant differences between group of extubation success and extubation failure of critically ill COVID-19 patients, as reported in Table 1.

In the mortality status, we found no significant differences between group of survived and non-survived of critically ill COVID-19 patients, as reported in Table 2.

Radiographic findings were reported by Radiologists in Cipto Mangunkusumo General (National Tertiary Referral Hospital). Chest X-ray findings in critically ill COVID-19 patients predominantly showed bilateral consolidation (*n* = 27) with severely affected right lung (*n* = 19), as shown in Appendix 3. The laboratory profiles of critically ill COVID-19 patients based on extubation and mortality status are shown in Tables 3 and 4, respectively. In the extubation status, we found no significant differences between group of extubation success and extubation failure of critically ill COVID-19 patients, as reported in Table 3.

In the mortality status, we found a significant difference of D-dimer between group of survived and non-survived of critically ill COVID-19 patients (12,646 vs. 1800 µg/L; *p* = .027), as reported in Table 4.

In the analysis of severely and less severely affected right and left lung, there were significantly lower BALF CD4 T-cells count in the right lung of critically ill

Table 1. Clinical characteristics of critically ill COVID-19 patients on extubation status.

Variable	Extubation success (n = 5)	Extubation failure (n = 23)	p
Age, years	64 (±8)	61 (±11.5)	.550
Gender (male, n, %)	3 (60)	13 (56)	1
BMI (kg/m ²), mean (±SD)	26.53 (±4)	26.85 (±3.42)	.855
Smoker, n (%)	0 (0)	7 (30)	.154
Severely affected lung (right, n, %)	3 (60)	16 (70)	.678
Comorbidities			
Diabetes mellitus, n (%)	3 (60)	13 (56)	.887
Hypertension, n (%)	3 (60)	11 (48)	.662
Chronic kidney disease, n (%)	1 (20)	9 (39)	.418
Scoring system			
APACHE II score, mean (±SD)	14.20 (±3.70)	16.30 (±5.15)	.397
mSOFA score, median (IQR)	8.00 (8.00–8.00)	9.00 (8.00–10.00)	.099
CCI, mean (±SD)	5.20 (±2.68)	4.57 (±2.10)	.565

SD: standard deviation; IQR: interquartile range; APACHE: acute physiology and chronic health evaluation; mSOFA: modified sequential organ failure assessment; CCI: comorbidity channel index.

Table 2. Clinical characteristics of critically ill COVID-19 patients on mortality status.

Variable	Survived (n = 6)	Non-survived (n = 22)	p
Age, years	63 (±8)	61 (±12)	.746
Gender (male, n, %)	3 (50)	13 (59)	1
BMI (kg/m ²), mean (±SD)	27.57 (±4.4)	26.58 (±3.24)	.542
Smoker, n (%)	0 (0)	7 (32)	.111
Severely affected lung (right, n, %)	4 (66)	15 (68)	.944
Comorbidities			
Diabetes mellitus, n (%)	4 (66)	12 (54)	.595
Hypertension, n (%)	4 (66)	10 (50)	.357
Chronic kidney disease, n (%)	1 (17)	9 (41)	.272
Scoring system			
APACHE II score, mean (±SD)	13.50 (±3.73)	16.60 (±5.07)	.178
mSOFA score, median (IQR)	8.00 (8.00–8.00)	9.00 (8.00–10.00)	.052
CCI, mean (±SD)	5.00 (±2.45)	4.59 (±2.15)	.691

SD: standard deviation; IQR: interquartile range; APACHE: acute physiology and chronic health evaluation; mSOFA: modified sequential organ failure assessment; CCI: comorbidity channel index.

COVID-19 patients (14 vs. 28 cells/μL; $p = .001$), as reported in Table 5.

Bronchoalveolar lavage fluid immunopathology in severely affected lung of critically ill COVID-19 pneumonia with extubation success and failure

Bronchoalveolar lavage fluid immunopathology profiles are shown in Tables 6 and 7. In the group of extubation failure, we found significantly lower BALF CD4 T-cells count (56 vs. 11 cells/μL; $p = .010$), as reported in Table 6.

Bronchoalveolar lavage fluid immunopathology in severely affected lung of survivors and non-survivors critically ill COVID-19 pneumonia

In the group of non-survivors, we found significantly lower BALF CD4 T-cells count (40 vs. 11 cells/μL; $p = .018$), as reported in Table 7.

Bronchoalveolar lavage fluid immunopathology biomarkers in less severely affected lung of critically ill COVID-19 are reported in Appendixes 1 and 2.

Discussion

The role of biomarkers has been suggested to guide clinicians for ventilator management in determining the potential risk of extubation failure and mortality [12]. This study implicates that the role of BALF CD4 T-cells as a biomarker is reliable for early management prediction in critically ill COVID-19 pneumonia patients (i.e. ventilator management, early tracheostomy and the need for aggressive treatment) which could shorten the length of hospital stays and to determine the readiness for extubation in patients who are at high risk for extubation failure.

Based on sample size calculation, 1010 patients were required in this study. Due to pandemic beta variant COVID-19 in Indonesia (November 2020 to January 2021), after the enrolment of 40 patients, an internal discussion was held, and the decision was to discontinue patients' enrolment. To our knowledge, only a few studies were conducted during the peak COVID-19 period analysing BALF immunopathology to assess extubation failure and mortality [46]. Additionally, this is the first study to perform early bronchoscopy after patients' intubation (average of

Table 3. Laboratory profiles of critically ill COVID-19 patients on extubation status.

Lab profiles	Extubation success (n = 5)	Extubation failure (n = 23)	p
Haemoglobin (g/dL), mean (±SD)	14.16 (±1.20)	13.84 (±2.83)	.808
Leukocytes ($\times 10^3$ cells/ μ L), mean (±SD)	15.15 (±4.82)	12.80 (±5.05)	.352
Neutrophils (%), mean (±SD)	85.66 (±4.60)	84.21 (±7.27)	.676
Lymphocytes (%), median (IQR)	11.30 (6.70–12.00)	9.90 (5.05–13.45)	.928
CRP (mg/L), mean (±SD)	191.38 (±155.83)	172.30 (±116.14)	.756
Ferritin (ng/mL), median (IQR)	2647.60 (1479.67–3510.22)	2333.00 (1259.55–3312.57)	.787
Albumin (g/dL), mean (±SD)	2.87 (±0.35)	3.18 (±0.49)	.194
D-dimer (μ g/L), median (IQR)	9560 (6890–18,160)	1880 (1385–8670)	.063
PaO ₂ /FiO ₂ ratio, median (IQR)	93.40 (47.60–95.54)	55.11 (48.65–92.74)	.653
Absolute CD4+ (cells/ μ L), mean (±SD)	229.60 (±132.851)	204.73 (±114.485)	.673
Serum IL-6 (pg/mL), median (IQR)	47.11 (32.65–48.14)	49.47 (27.30–89.84)	.529
Serum IL-17 (pg/mL), mean (±SD)	11.55 (±1.12)	11.88 (±0.78)	.756

CRP: C-reactive protein; PaO₂/FiO₂: the ratio of arterial oxygen partial pressure to fractional inspired oxygen; CD: cluster of differentiation; IL: interleukin.

Table 4. Laboratory profiles of critically ill COVID-19 patients on mortality status.

Lab profiles	Survived (n = 6)	Non-survived (n = 22)	p
Haemoglobin (g/dL), mean (±SD)	14.45 (±1.28)	13.74 (±2.86)	.566
Leukocytes ($\times 10^3$ cells/ μ L), mean (±SD)	14.50 (±4.59)	12.87 (±5.16)	.489
Neutrophils (%), mean (±SD)	84.21 (±5.43)	84.54 (±7.26)	.920
Lymphocytes (%), median (IQR)	11.65 (6.70–13.60)	9.45 (5.00–13.00)	.654
CRP (mg/L), median (IQR)	182.00 (109.20–291.00)	140.15 (81.80–250.00)	.758
Ferritin (ng/mL), mean (±SD)	2459.72 (±1970.76)	2636.88 (±1853.21)	.893
Albumin (g/dL), mean (±SD)	2.82 (±0.33)	3.21 (±0.48)	.076
D-dimer (μ g/L), median (IQR)	12,645 (6890–18,160)	1800 (1370–7400)	.027
PaO ₂ /FiO ₂ ratio, median (IQR)	70.50 (40.00–95.54)	56.55 (49.00–103.75)	.780
Absolute CD4+ (cells/ μ L), mean (±SD)	266.33 (±149.049)	193.05 (±103.009)	.176
Serum IL-6 (pg/mL), median (IQR)	47.62 (32.65–67.04)	45.68 (27.16–88.90)	.955
Serum IL-17 (pg/mL), mean (±SD)	11.67 (±1.05)	11.87 (±0.80)	.628

CRP: C-reactive protein; PaO₂/FiO₂: the ratio of arterial oxygen partial pressure to fractional inspired oxygen; CD: cluster of differentiation; IL: interleukin.

Table 5. BALF immunopathology biomarkers in right and left lung.

Biomarkers	Right lung (n = 28)	Left lung (n = 28)	p
sTREM (ng/mg protein), median (IQR)	1.23 (0.89–2.88)	1.16 (0.82–2.46)	.179
Alveolar macrophage			
% (Macrophage cells/CD45+, HLA-DR+, CD11b+), median (IQR)	7.30 (2.75–10.20)	6.00 (2.45–10.05)	.909
MFI CD169, median (IQR)	701 (347.50–858.50)	610 (347–948.50)	.855
IL-6 (pg/mg protein), median (IQR)	332.02 (153.29–612.24)	270.41 (124.28–514.67)	.187
IL-17 (pg/mg protein), median (IQR)	7.56 (5.32–9.77)	5.68 (4.54–9.29)	.072
CD4 T-cells (cells/ μ L), median (IQR)	14 (8.5–24)	28 (15.5–53.50)	.001
Foxp3+ CD25+/CD4 (%) (median (IQR))	19.64 (13.09–30.67)	14.38 (10.93–25.95)	.524
Caspase 3 (ng/mg protein), median (IQR)	0.82 (0.44–1.44)	0.70 (0.40–1.27)	.305
SP-A (ng/mg protein), median (IQR)	15.26 (9.32–19.48)	13.07 (10.97–14.66)	.194

Table 6. BALF immunopathology biomarkers of severely affected lung on extubation status.

Biomarkers	Extubation success (n = 5)	Extubation failure (n = 23)	p
sTREM (ng/mg protein), median (IQR)	0.92 (0.3–3.71)	1.23 (0.35–10.72)	.453
Alveolar macrophage			
% (Macrophage cells/CD45+, HLA-DR+, CD11b+), mean (±SD)	8.4 (±8.44)	6.98 (±4.23)	.584
MFI CD169, median (IQR)	672 (308–738)	775 (217–7765)	.384
IL-6 (pg/mg protein), median (IQR)	173.52 (60.27–378.63)	337.65 (34.7–1689.95)	.197
IL-17 (pg/mg protein), median (IQR)	6.11 (3.83–10.05)	6.3 (1.86–29.31)	.976
CD4 T-cells (cells/ μ L), median (IQR)	56 (16–102)	11 (4–70)	.010
Foxp3+ CD25+/CD4 (%) (median (IQR))	21.38 (6.71–28.13)	16.35 (4.5–81.4)	.976
Caspase 3 (ng/mg protein), median (IQR)	0.99 (0.56–2.54)	0.82 (0.14–5.33)	.384
SP-A (ng/mg protein), mean (±SD)	12.79 (±3.62)	14.86 (±5.43)	.427

MFI: mean fluorescence intensity.

4 h, SD = 0.82) in severely and less severely affected lung to enumerate BALF immunopathology. Most BALF studies during pandemic COVID-19 have analysed cellularity characteristics [47–50] (i.e. CD4 T-cells, CD8 T-cells, macrophages). However, no data are available for combined BALF immunopathology biomarkers (i.e. sTREM, IL-6, IL-17, SP-A and Caspase-3).

Based on extubation and mortality status, we also observed clinical characteristics and laboratory profiles of critically ill COVID-19 pneumonia patients. D-dimer levels were significantly higher in the group of survived rather than non-survived. The role of elevated D-dimer in critically ill COVID-19 has been associated with disease severity and mortality [51]. Other studies have found elevated

Table 7. BALF immunopathology biomarkers of severely affected lung on mortality status.

Biomarkers	Survivors (n = 6)	Non-survivors (n = 22)	p
sTREM (ng/mg protein), median (IQR)	1.26 (0.3–3.71)	1.17 (0.37–10.72)	.780
Alveolar macrophage			
% (Macrophage cells/CD45+, HLA-DR+, CD11b+), mean (\pm SD)	8.7 (\pm 7.59)	6.8 (\pm 4.28)	.443
MFI CD169, median (IQR)	512.5 (308–738)	775 (217–7765)	.218
IL-6 (pg/mg protein), median (IQR)	218.25 (60.27–378.63)	375.51 (34.7–1689.95)	.218
IL-17 (pg/mg protein), median (IQR)	7.8 (3.83–29.31)	6.1 (1.86–27.17)	.467
CD4 T-cells (cells/ μ L), median (IQR)	40 (14–102)	11 (4–70)	.018
Foxp3+ CD25+/CD4 (%), median (IQR)	17.8 (6.71–28.13)	16.93 (4.5–81.4)	.654
Caspase 3 (ng/mg protein), median (IQR)	1.4 (0.56–5.33)	0.77 (0.14–2.91)	.117
SP-A (ng/mg protein), mean (\pm SD)	13.76 (\pm 4.0)	14.7 (\pm 5.5)	.701

MFI: mean fluorescence intensity.

D-dimer to be associated with extubation failure and death [52,53]. Our study found a different result for elevated D-dimer probably due to small sample size, no serial D-dimer evaluation, and selection bias.

Several studies reported bilateral lung consolidation as frequent abnormalities findings in COVID-19 patients [54,55]. In this study, the majority of patients' chest radiographs were characterized by bilateral diffuse consolidation, with the right lung being the most severely affected. Differences in anatomical structure led to a higher likelihood of foreign particles and pathogens entering the lower respiratory tract of the right lung [56–58]. Hence, the severity of inflammation will predominate in the right lung (marked by significantly low BALF CD4 T-cells count), as shown in this study. In the severe inflammation process, CD4 T-cells will differentiate into several subsets, including Tregs [59]. This study also displayed a high level of Tregs in extubation success and survived groups, albeit statistically not significant. This result indicates the vital function of Tregs as an immune suppressor in helping the adaptive immune process [60], and its role in the lung epithelial regeneration and surfactant production [61], as well as the potential effect on the resolution of the lung [62]. Future studies are required to investigate the detailed mechanisms of Tregs and the association with extubation success or extubation failure, which may better explain this present study.

Limited data are available for assessing extubation failure in critically ill COVID-19 pneumonia patients [7]. Information focussing on extubation failure in severe pneumonia patients has been reported by Yu et al. [10] Critically ill COVID-19 pneumonia patients need more prolonged intubation time [63], and Ionescu et al. [7] reported extubation failure in critically ill COVID-19 pneumonia was strongly associated with hospital mortality. Length of stays, requirement of tracheostomy and mortality were associated with extubation failure [64].

A BALF study by Saris et al. found that critically COVID-19 pneumonia patients showed a reduced levels of BALF CD4 T-cells count in non-survivor compared to

the survivor group [46]. This study reports a significant low levels of BALF CD4 T-cells count in severely affected lung of critically ill COVID-19 pneumonia patients in extubation failure and non-survived group.

It is recommended for clinicians to evaluate BALF in severe pneumonia patients [65]. However, limited BALF immunopathology studies were found in severe pneumonia patients. Meta-analysis by Zeng et al. [66] reported serum immunopathology in critically ill COVID-19 pneumonia is associated with disease severity and high mortality. Sputum and BALF inflammatory biomarkers, IL-6, IL-8, IL-10, IL-17, IL-1 β , IL-1ra, TNF- α and IFN- γ , have been reported in several studies [1,13]. Bronchoalveolar lavage fluid immunopathology biomarkers have been described to predict clinical outcomes in COVID-19 pneumonia patients [67]. Eight BALF immunopathology biomarkers were assessed in this study, sTREM-1, alveolar macrophage, IL-6, IL-17, CD4 T-cells, Tregs, SP-A and Caspase-3. Based on the immunopathophysiology, the selection of eight biomarkers in this study (sTREM-1, alveolar macrophage amount and function, IL-6, IL-17, CD4 T-cells, Tregs, SP-A and Caspase-3) could provide a better comprehensive in explaining the role of local innate and adaptive immune responses and its association with extubation failure and mortality. The presence of infectious disease can be marked by the presence of sTREM-1 [68]. Alveolar macrophage as a phagocyte plays a role in homeostasis, body protection, response to foreign particles, and tissue regeneration. Additionally, alveolar macrophage function regulates local immunology, especially lung immunity. The interaction of each cell will mediate the immune response. Alveolar macrophages will secrete cytokines and eicosanoid for neutrophil polymorphonuclear recruitment [69]. Kooguchi et al. [22] found that downregulation of alveolar macrophages will increase the severity of lung injury and mortality of pneumonia, and a study conducted by Puren et al. [23] found that a decrease/increase in cytokine (IL-6 and IL-17) levels are associated with severity of infection and inflammation. A study by Jedynak

et al. [70] found that an increase in lung infection will stimulate sTREM-1 to activate monocyte and neutrophil and produce more inflammatory cytokines. Insufficient protection of alveolar macrophages to the infection will initiate adaptive immunity and activate lymphocytes (T cells), which then induce apoptosis (upregulated Caspase-3 and downregulated CD4 T-cells) [71]. Bielosludtseva et al. reported that reduction of T cell levels is associated with high mortality in pneumonia [30]. Additionally, a study by Shi et al. [38], reported that an increase in Caspase-3 is associated with increased alveolar epithelial apoptosis. Persistent severe lung injury will dysregulate lung epithelium regeneration (Tregs) and reduce surfactant production [61]. A study by Mukherjee et al. [36] reported that downregulated surfactant (SP-A) production will cause lung atelectasis and gas exchange disturbance. Previous studies explained that the reduction of immune cells and mediators will cause individual be more prone to diseases (especially infectious diseases) and/or will aggravate the existed disease condition [72].

This study reports significantly low levels of BALF CD4 T-cells count in extubation failure ($p = .010$) and non-survived ($p = .018$) critically ill COVID-19 pneumonia patients. Jiang et al. [8] reported a significantly low serum CD4 T-cells count in non-survived COVID-19 pneumonia patients. Vedder et al. [73] described normal BALF CD4 T-cells in ventilated COVID-19 pneumonia patients (BALF was collected within two days after mechanical ventilation), whereas in this study BALF was collected in an average of 4 h ($SD = 0.82$) after patients' intubation. No significant differences were found in other BALF immunopathology biomarkers (sTREM-1, alveolar macrophage, IL-6, IL-17, Tregs, SP-A and Caspase-3) for both groups (extubation failure and mortality) in this study.

Some study limitations included: (1) the small number of study subjects may result in other biomarkers that were not statically significant. The lack of statistical significance in some BALF biomarkers may be explained by our current sample size being too small to detect the difference. Therefore, future studies with larger sample size are suggested to investigate the differences in other BALF biomarkers. (2) Chest CT scan imaging was not performed; hence, we could not assess other abnormalities that might be presented in the study patients. (3) No serial evolution time for BALF biomarkers to assess the dynamic changes.

Conclusions

This study reports the association of low BALF CD4 T-cells count with early extubation failure and mortality

in the severely affected lung of critically ill COVID-19 pneumonia patients. BALF CD4 T-cells count evaluation of severely affected lung can be an option to clinicians for assessment of early extubation failure and mortality in critically ill COVID-19 pneumonia patients.

Acknowledgements

The authors thank all patients and their families, and health-care workers involved in this study who with full encouragement are consistently providing care for patients in COVID-19 pandemic. Special thanks to Dita Aditiansih, MD, Adhrie Sugiarto, MD, and Sidharta Kusuma Manggala, MD (Intensivists); Dewi Wulandari, MD, Eka (Clinical Pathologists); Saras and team (Integrated Laboratory Medical Faculty, Universitas Indonesia); Hadiki Habib, MD and Fidiayi Toni, MD (Internists/Emergency physicians); I Putu Eka Krishna Wijaya, MD, Ahmad Nur Aulia, MD, Nova Bornida Fauzi, MD, Muhammad Adriyanto, MD, Sudirman Fakhruddin Masse, MBBS and Indah Septiani (Division of Respirology and Critical Illness, Internal Medicine Department); Utami (Research Co-ordinator unit) for their contributions in conducting this study.

Registration: The study was registered at UMIN Clinical Trials Registry (UMIN-CTR) (registration number UMIN000046236, last modified date: 2022/04/18), accessible at: https://center6.umin.ac.jp/cgi-open-bin/ctr_e/ctr_view.cgi?recptno=R000049197

Author contributions

G.S. performed material preparation and data collection. G.S., C.M.R., S.K.S., Z.A., I.R., T.L., E.H., K.H. and H.W. contributed to the study conception, design and analysis. G.S. wrote the first draft of the manuscript. All authors read and approved the final manuscript.

Ethical approval

This study has been approved by Ethics Committee Universitas Indonesia (approval number: KET-171/UN2.F1/ETIK/PPM.00.02/2020) and Ethics Committee of Health Research, Cipto Mangunkusumo Hospital. Informed consent was approved by Ethics Committee (approval number: 0995/rev00/KEP/2020).

Patient consent

Informed consent for bronchoscopy was signed by patient's family member (patients were intubated).

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

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 subjects.

Funding

The author(s) reported there is no funding associated with the work featured in this article.

References

- [1] Fernandez-Botran R, Uriarte SM, Arnold FW, et al. Contrasting inflammatory responses in severe and non-severe community-acquired pneumonia. *Inflammation*. 2014;37(4):1158–1166.
- [2] Coma E, Méndez-Boo L, Mora N, et al. Divergences on expected pneumonia cases during the COVID-19 epidemic in Catalonia: a time-series analysis of primary care electronic health records covering about 6 million people. *BMC Infect Dis*. 2021;21(1):283.
- [3] García LF. Immune response, inflammation, and the clinical spectrum of COVID-19. *Front Immunol*. 2020; 11:1441.
- [4] Wendel Garcia PD, Fumeaux T, Guerci P, et al. Prognostic factors associated with mortality risk and disease progression in 639 critically ill patients with COVID-19 in Europe: initial report of the international RISC-19-ICU prospective observational cohort. *EClinicalMedicine*. 2020;25:100449.
- [5] Alharthy A, Aletreby W, Faqih F, et al. Clinical characteristics and predictors of 28-day mortality in 352 critically ill patients with COVID-19: a retrospective study. *J Epidemiol Glob Health*. 2021;11(1):98–104.
- [6] Gamberini L, Tonetti T, Spadaro S, 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.
- [7] Ionescu F, Zimmer MS, Petrescu I, et al. Extubation failure in critically ill COVID-19 patients: risk factors and impact on in-hospital mortality. *J Intensive Care Med*. 2021;36(9):1018–1024.
- [8] Jiang Y, Abudurexiti S, An M-M, et al. Risk factors associated with 28-day all-cause mortality in older severe COVID-19 patients in Wuhan, China: a retrospective observational study. *Sci Rep*. 2020;10(1): 22369.
- [9] Leoni MLG, Lombardelli L, Colombi D, et al. Prediction of 28-day mortality in critically ill patients with COVID-19: development and internal validation of a clinical prediction model. *PLOS One*. 2021;16(7): e0254550.
- [10] Yu H, Luo J, Ni Y, et al. Early prediction of extubation failure in patients with severe pneumonia: a retrospective cohort study. *Biosci Rep*. 2020;40: BSR20192435.
- [11] Ciaccio M, Agnello L. Biochemical biomarkers alterations in coronavirus disease 2019 (COVID-19). *Diagnosis*. 2020;7(4):365–372.
- [12] Raventós AA, Serpa Neto A. Biomarkers to guide ventilation management and readiness for extubation. *Am J Respir Crit Care Med*. 2021;203(10):1211–1212.
- [13] Paats MS, Bergen IM, Hanselaar WEJJ, et al. Local and systemic cytokine profiles in nonsevere and severe community-acquired pneumonia. *Eur Respir J*. 2013; 41(6):1378–1385.
- [14] Ramirez P, Kot P, Marti V, et al. Diagnostic implications of soluble triggering receptor expressed on myeloid cells-1 in patients with acute respiratory distress syndrome and abdominal diseases: a preliminary observational study. *Crit Care*. 2011;15(1):R50.
- [15] Shi J-X, Li J-S, Hu R, et al. Diagnostic value of sTREM-1 in bronchoalveolar lavage fluid in ICU patients with bacterial lung infections: a bivariate meta-analysis. *PLOS One*. 2013;8(5):e65436.
- [16] Al-Omari B, McMeekin P, Allen AJ, et al. Systematic review of studies investigating ventilator associated pneumonia diagnostics in intensive care. *BMC Pulm Med*. 2021;21(1):196.
- [17] Huh JW, Lim C-M, Koh Y, et al. Diagnostic utility of the soluble triggering receptor expressed on myeloid cells-1 in bronchoalveolar lavage fluid from patients with bilateral lung infiltrates. *Crit Care*. 2008;12(1):R6.
- [18] Gibot S, Cravoisy A, Levy B, et al. Soluble triggering receptor expressed on myeloid cells and the diagnosis of pneumonia. *N Engl J Med*. 2004;350(5):451–458.
- [19] Guillon A, Arafa El, Barker KA, et al. Pneumonia recovery reprograms the alveolar macrophage pool. *JCI Insight*. 2020;5(4):e133042.
- [20] Knapp S, Leemans JC, Florquin S, et al. Alveolar macrophages have a protective antiinflammatory role during murine pneumococcal pneumonia. *Am J Respir Crit Care Med*. 2003;167(2):171–179.
- [21] Liao M, Liu Y, Yuan J, et al. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. *Nat Med*. 2020;26(6):842–844.
- [22] Kooguchi K, Hashimoto S, Kobayashi A, et al. Role of alveolar macrophages in initiation and regulation of inflammation in *Pseudomonas aeruginosa* pneumonia. *Infect Immun*. 1998;66(7):3164–3169.
- [23] Puren AJ, Feldman C, Savage N, et al. Patterns of cytokine expression in community-acquired pneumonia. *Chest*. 1995;107(5):1342–1349.
- [24] Montón C, Torres A, El-Ebiary M, et al. Cytokine expression in severe pneumonia: a bronchoalveolar lavage study. *Crit Care Med*. 1999;27:1745–1753.
- [25] Wiedermann FJ, Lederer W. Inflammatory factors in alveolar lavage fluid from severe COVID-19 pneumonia: PCT and IL-6 in epithelial lining fluid. *Open Med*. 2021;16(1):1132–1133.
- [26] Wonnemberg B, Jungnickel C, Honecker A, et al. IL-17A attracts inflammatory cells in murine lung infection with *P. aeruginosa*. *Innate Immun*. 2016;22(8): 620–625.
- [27] Ritchie ND, Ritchie R, Bayes HK, et al. IL-17 can be protective or deleterious in murine pneumococcal pneumonia. *PLoS Pathog*. 2018;14(5):e1007099.

- [28] Orlov M, Dmyterko V, Wurfel MM, et al. Th17 cells are associated with protection from ventilator associated pneumonia. *PLOS One*. 2017;12(8):e0182966.
- [29] Tsai HC, Velichko S, Hung LY, et al. IL-17A and Th17 cells in lung inflammation: an update on the role of Th17 cell differentiation and IL-17R signaling in host defense against infection. *Clin Dev Immunol*. 2013;2013:267971.
- [30] Bielosludtseva K, Pertseva T, Kyreeva T. CD4 as a predictor of systemic inflammatory response and death at severe community-acquired pneumonia. *Eur Respir J*. 2013;42:4366.
- [31] Jambo KC, Sepako E, Fullerton DG, et al. Bronchoalveolar CD4+ T cell responses to respiratory antigens are impaired in HIV-infected adults. *Thorax*. 2011;66(5):375–382.
- [32] Park H, Li Z, Yang XO, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol*. 2005;6(11):1133–1141.
- [33] Neill DR, Fernandes VE, Wisby L, et al. T regulatory cells control susceptibility to invasive pneumococcal pneumonia in mice. *PLoS Pathog*. 2012;8(4):e1002660.
- [34] Wang Y, Zheng J, Islam MS, et al. The role of CD4(+)/FoxP3(+) regulatory T cells in the immunopathogenesis of COVID-19: implications for treatment. *Int J Biol Sci*. 2021;17(6):1507–1520.
- [35] Adamzik M, Broll J, Steinmann J, 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(10):1743–1751.
- [36] Mukherjee S, Giamberardino C, Thomas JM, et al. Surfactant protein a modulates induction of regulatory T cells via TGF- β . *J Immunol*. 2012;188(9):4376–4384.
- [37] Du X, Meng Q, Sharif A, 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(2):164–172.
- [38] Shi S, Liu X, Li H. Downregulation of caspase-3 alleviates *Mycoplasma pneumoniae*-induced apoptosis in alveolar epithelial cells. *Mol Med Rep*. 2017;16(6):9601–9606.
- [39] Kosai K, Seki M, Tanaka A, et al. Increase of apoptosis in a murine model for severe pneumococcal pneumonia during influenza a virus infection. *Jpn J Infect Dis*. 2011;64(6):451–457.
- [40] Baughman RP, Sternberg RI, Hull W, et al. Decreased surfactant protein A in patients with bacterial pneumonia. *Am Rev Respir Dis*. 1993;147(3):653–657.
- [41] Mandell LA, Wunderink RG, Anzueto A, 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):S27–S72.
- [42] Feng F, Jiang Y, Yuan M, et al. Association of radiologic findings with mortality in patients with avian influenza H7N9 pneumonia. *PLOS One*. 2014;9(4):e93885.
- [43] Hunninghake GW, Gadek J, Kawanami O, et al. Inflammatory and immune processes in the human lung in health and disease: evaluation by bronchoalveolar lavage. *Am J Pathol*. 1979;97(1):149–206.
- [44] Baldwin DR, Wise R, Andrews JM, et al. Microlavage: a technique for determining the volume of epithelial lining fluid. *Thorax*. 1991;46(9):658–662.
- [45] Nayak DK, Mendez O, Bowen S, et al. Isolation and in vitro culture of murine and human alveolar macrophages. *J Vis Exp*. 2018.
- [46] Saris A, Reijnders TDY, Nossent EJ, et al. Distinct cellular immune profiles in the airways and blood of critically ill patients with COVID-19. *Thorax*. 2021;76(10):1010–1019.
- [47] Baron A, Hachem M, Tran Van Nhieu J, et al. Bronchoalveolar lavage in patients with COVID-19 with invasive mechanical ventilation for acute respiratory distress syndrome. *Ann Am Thorac Soc*. 2021;18(4):723–726.
- [48] Dentone C, Vena A, Loconte M, et al. Bronchoalveolar lavage fluid characteristics and outcomes of invasively mechanically ventilated patients with COVID-19 pneumonia in Genoa, Italy. *BMC Infect Dis*. 2021;21(1):353.
- [49] Gelarden I, Nguyen J, Gao J, et al. Comprehensive evaluation of bronchoalveolar lavage from patients with severe COVID-19 and correlation with clinical outcomes. *Hum Pathol*. 2021;113:92–103.
- [50] Voiriot G, Fajac A, Lopinto J, et al. Bronchoalveolar lavage findings in severe COVID-19 pneumonia. *Intern Emerg Med*. 2020;15(7):1333–1334.
- [51] Yao Y, Cao J, Wang Q, 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.
- [52] Cerda-Mancillas MC, Santiago-Germán D, Andrade-Bravo B, et al. D-Dimer as a biomarker of severity and adverse outcomes in patients with community acquired pneumonia. *Arch Med Res*. 2020;51(5):429–435.
- [53] King CS, Sahjwani D, Brown AW, et al. Outcomes of mechanically ventilated patients with COVID-19 associated respiratory failure. *PLOS One*. 2020;15(11):e0242651.
- [54] Wong HYF, Lam HYS, Fong AH-T, et al. Frequency and distribution of chest radiographic findings in patients positive for COVID-19. *Radiology*. 2020;296(2):E72–E78.
- [55] Yasin R, Gouda W. Chest X-ray findings monitoring COVID-19 disease course and severity. *Egypt J Radiol Nucl Med*. 2020;51(1):193.
- [56] Sinha V, Chhaya V, Barot DS, et al. Foreign body in tracheobronchial tree. *Indian J Otolaryngol Head Neck Surg*. 2010;62(2):168–170.
- [57] Huffnagle GB, Dickson RP. The bacterial microbiota in inflammatory lung diseases. *Clin Immunol*. 2015;159(2):177–182.
- [58] Chaudhry R, Bordoni B. *StatPearls*. StatPearls Publishing; 2021.
- [59] Martinez-Sanchez ME, Huerta L, Alvarez-Buylla ER, et al. Role of cytokine combinations on CD4+ T cell differentiation, partial polarization, and plasticity: continuous network modeling approach. *Front Physiol*. 2018;9:877.

- [60] Corthay A. How do regulatory T cells work? *Scand J Immunol.* 2009;70(4):326–336.
- [61] Mock JR, Garibaldi BT, Aggarwal NR, et al. Foxp3+ regulatory T cells promote lung epithelial proliferation. *Mucosal Immunol.* 2014;7(6):1440–1451.
- [62] Norton DL, Ceppe A, Tune MK, et al. Bronchoalveolar Tregs are associated with duration of mechanical ventilation in acute respiratory distress syndrome. *J Transl Med.* 2020;18(1):427.
- [63] Burns GD, Phillips J, Pangilinan LP, et al. Time to extubation with and without COVID-19 pneumonia. *Respir Care.* 2021;66:3607976.
- [64] Funk G-C, Anders S, Breyer M-K, et al. Incidence and outcome of weaning from mechanical ventilation according to new categories. *Eur Respir J.* 2010;35(1):88–94.
- [65] Davidson KR, Ha DM, Schwarz MI, et al. Bronchoalveolar lavage as a diagnostic procedure: a review of known cellular and molecular findings in various lung diseases. *J Thorac Dis.* 2020;12(9):4991–5019.
- [66] Zeng F, Huang Y, Guo Y, et al. Association of inflammatory markers with the severity of COVID-19: a meta-analysis. *Int J Infect Dis.* 2020;96:467–474.
- [67] Pandolfi L, Fossali T, Frangipane V, et al. Bronchoalveolar inflammation in COVID-19 patients: a correlation with clinical outcome. *BMC Pulm Med.* 2020;20(1):301.
- [68] Cao C, Gu J, Zhang J. Soluble triggering receptor expressed on myeloid cell-1 (sTREM-1): a potential biomarker for the diagnosis of infectious diseases. *Front Med.* 2017;11(2):169–177.
- [69] Gordon SB, Read RC. Macrophage defences against respiratory tract infections. *Br Med Bull.* 2002;61:45–61.
- [70] Jedynek M, Siemiatkowski A, Mroczko B, et al. Soluble TREM-1 serum level can early predict mortality of patients with sepsis, severe sepsis and septic shock. *Arch Immunol Ther Exp.* 2018;66(4):299–306.
- [71] Kemp K, Bruunsgaard H, Skinhøj P, et al. Pneumococcal infections in humans are associated with increased apoptosis and trafficking of type 1 cytokine-producing T cells. *Infect Immun.* 2002;70(9):5019–5025.
- [72] Reynolds JH, McDonald G, Alton H, et al. Pneumonia in the immunocompetent patient. *Br J Radiol.* 2010; 83(996):998–1009.
- [73] Vedder V, Schildgen V, Lüsebrink J, et al. Differential cytology profiles in bronchoalveolar lavage (BAL) in COVID-19 patients: a descriptive observation and comparison with other corona viruses, influenza virus, *Haemophilus influenzae*, and *Pneumocystis jirovecii*. *Medicine.* 2021;100(1):e24256.

Appendix 1

BALF immunopathology biomarkers in critically ill COVID-19 pneumonia (less severely affected lung).

Biomarkers	Extubation success (<i>n</i> = 5)	Extubation failure (<i>n</i> = 23)	<i>p</i>
sTREM (ng/mg protein), median (IQR)	1.62 (1.09–2.00)	1.22 (0.84–2.28)	.697
Alveolar macrophage % (Macrophage cells/CD45+, HLA-DR+, CD11b+), median (IQR)	8.00 (5.10–9.10)	5.70 (2.70–10.80)	.472
MFI CD169, median (IQR)	598 (385–888)	628 (347.50–961.50)	.857
IL-6 (pg/mg protein), mean (±SD)	253.30 (±134.58)	393.04 (±315.57)	.346
IL-17 (pg/mg protein), median (IQR)	10.99 (10.58–11.08)	5.87 (4.54–8.56)	.051
CD4 (cell/μL), median (IQR)	37 (32–52)	23 (13.50–39)	.352
Foxp3+ CD25+/CD4 (%), median (IQR)	19.13 (13.86–19.23)	15.49 (12.24–33.18)	.697
Caspase 3 (ng/mg protein), median (IQR)	0.97 (0.57–1.05)	0.67 (0.36–1.26)	.881
SP-A (ng/mg protein), median (IQR)	13.03 (13.00–13.49)	12.56 (9.79–16.45)	.529

MFI: mean fluorescence intensity.

Appendix 2

BALF immunopathology biomarkers in critically ill COVID-19 pneumonia (less severely affected lung).

Biomarkers	Survivors (<i>n</i> = 6)	Non-survivors (<i>n</i> = 22)	<i>p</i>
sTREM (ng/mg protein), median (IQR)	1.81 (1.09–2.09)	1.10 (0.81–2.42)	.502
Alveolar macrophage % (Macrophage cells/CD45+, HLA-DR+, CD11b+), median (IQR)	8.55 (5.10–10.90)	5.55 (2.60–10.70)	.275
MFI CD169, median (IQR)	491.50 (341–888)	649.50 (354–1007)	.538
IL-6 (pg/mg protein), median (IQR)	302.07 (145.26–425.74)	310.77 (121.75–575.43)	.780
IL-17 (pg/mg protein), median (IQR)	11.04 (10.58–12.58)	5.82 (4.28–8.00)	.016
CD4 (cell/μL), median (IQR)	34.50 (26–52)	21.50 (13–48)	.370
Foxp3+ CD25+/CD4 (%), median (IQR)	16.50 (13.84–19.28)	17.81 (10.97–37.91)	.576
Caspase 3 (ng/mg protein), median (IQR)	1.01 (0.57–1.07)	0.66 (0.33–1.09)	.370
SP-A (ng/mg protein), mean (±SD)	16.78 (±7.76)	13.01 (±5.24)	.170

MFI: mean fluorescence intensity.

Appendix 3

Radiographic findings in critically ill COVID-19 pneumonia.

	<i>n</i> = 28 (%)
Chest X-ray	
Severely affected lung	
Right	19 (68)
Left	9 (32)
Baseline features	
Bilateral diffuse consolidation	27 (96)
Bilateral diffuse infiltrate	1 (3.5)
Pleural effusion	1 (3.5)
Pulmonary Nodules	2 (7)