ORIGINAL ARTICLE

ASSOCIATION BETWEEN FRACTION AND RATIO OF CD4/CD8 BRONCHOALVEOLAR LAVAGE FLUID TOWARD EXTUBATION STATUS AND MORTALITY STATUS OF PNEUMONIA SEVERE PATIENTS IN DR. CIPTO MANGUNKUSUMO NATIONAL GENERAL HOSPITAL, INDONESIA

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ABSTRACT

Background: Extubating failure due to severe pneumonia increases morbidity and mortality. Systemic adaptive immunity, T lymphocyte cells CD4/CD8 in blood, has special role as a mortality predictor in severe pneumonia. Further study still needed to evaluate local adaptive immunity through bronchoalveolar lavage cellular examination in both lungs.
Objective: The aim of this study was to find out the differences between T lymphocytes CD4/CD8 in both lungs based on extubating status and mortality status.
Methods: We performed a cohort prospective study of 40 patients with severe pneumonia whom underwent endotracheal intubation and bronchoscopy hospitalized in intensive care unit between November 2020 to January 2021 in Dr. Cipto Mangunkusumo National General Hospital. Primary data was taken and analyzed using univariate and bivariate to investigate mean or median differences with unpaired t-test for normal numeric distribution data and Mann-Whitney test for abnormal distribution numeric data.
Result: The proportion of extubating failure was 80% and mortality rate was 75%. There were significantly different results of BALF CD4 T cells lymphocyte fraction in severe pneumonia group of patients based on extubating status (p=0,006) and mortality status (p=0,002). Blood CD4 T cells lymphocyte fraction and blood CD4/CD8 T cells lymphocyte ratio were found significantly higher in the successfully extubating group of patients compared to extubating failure group of patients; and also, significantly higher in survived group of patients compared to mortality group of patients with pneumonia severe.
Conclusion: Fraction of CD4 BALF in severely injured pneumonia lungs group of patients who had successful intubation processes were statistically different compared to the group of patients with unsuccessful extubating. Fraction of CD4 BALF were also found statistically different in the group of patients who were survived compared to the group of patients who were passed away.
Key Words: Local Adaptive Immunity; Subset T Lymphocyte; CD4 cells; CD8 cells; Bronchoalveolar Lavage; Extubating failure; Severe Pneumonia

ABSTRAK
Latar Belakang: Kegagalan ekstubasi akibat pneumonia berat meningkatkan morbiditas dan mortalitas. Imunitas adaptif sistemik berupa fraksi dan rasio sel T limfosit CD4/CD8 darah memiliki peranan penting sebagai prediktor lemah mortalitas. Dibutuhkan studi lanjutan untuk mengetahui imunitas adaptif lokal melalui Bronchoalveolar Lavage (BAL) pada kedua paru.
Tujuan: Mengetahui perbedaan kadar dan rasio sel T limfosit CD4/CD8 Bronchoalveolar Lavage sesuai status ekstubasi dan status mortalitas pada pneumonia berat.
Metode: Penelitian ini menggunakan desain kohort prospektif pada 40 pasien pneumonia berat. Data primer diambil dari pasien yang terintubasi dan menjalani tindakan bronkoskopi di perawatan IGD dan ruang intensif RSCM sejak November 2020 hingga Januari 2021. Analisa univariat dan bivariat dengan uji beda rerata digunakan pada data skala numerik dengan sebaran normal dan uji Mann Whitney dengan sebaran tidak normal.
Hasil: Proporsi gagal ekstubasi sebesar 80% dan proporsi mortalitas sebesar 75%. Terdapat perbedaan bermakna pada fraksi sel T limfosit CD4 BAL pada paru cedera berat kelompok berhasil ekstubasi dan gagal ekstubasi (p=0,006); kelompok pasien hidup dan meninggal (p=0,002). Fraksi CD4 darah dan rasio CD4/CD8 darah ditemukan lebih tinggi secara bermakna pada kelompok berhasil ekstubasi dibandingkan dengan gagal ekstubasi; juga ditemukan lebih tinggi pada kelompok yang hidup dibandingkan yang meninggal.
Kesimpulan: Fraksi CD4 BAL pada paru cedera berat berbeda secara statistik bermakna lebih tinggi pada kelompok pasien berhasil ekstubasi dibandingkan dengan kelompok pasien gagal ekstubasi dan kelompok pasien hidup dibandingkan dengan kelompok pasien meninggal.
Kata Kunci: Imunopatologi Lokal Adaptif; Subset T Limfosit; CD4; CD8; Gagal Ekstubasi; Pneumonia Berat

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INTRODUCTION

Pneumonia is an acute lower respiratory tract infection of the lung parenchyma. Patients with severe pneumonia require intensive care admission and have high mortality and extubating failure risk. The American Thoracic Society (ATS) states that to fulfill the classification of severe pneumonia requires one of two major criteria (require mechanical ventilation or septic shock with vasopressors) and at least equal to three minor criteria.[1] In 2015, WHO stated that there were 920.136 deaths due to pneumonia.[2,3] Currently, the world is facing a cluster of pneumonia due to COVID-19 (Corona Virus Disease 2019). As of 20 December 2020, the number of cases has reached more than 75 million people and cause mortality for over 1,6 million deaths globally.[4] A systematic review shows the existence of various predictors of poor prognosis due to severe pneumonia, including COVID-19, are : (1) advanced age, (2) comorbid diseases (such as hypertension, cardiovascular disease, diabetes mellitus, lung disease, (3) obesity (excess adipose tissue), (4) the presence of secondary infection, (5) elevated blood inflammatory markers.[5] Extubating failure due to severe pneumonia increases morbidity and mortality.[6]

Cellular adaptive immunity has specific role in pathophysiology of severe pneumonia related to its mortality and extubating failure rate.[7] Several immunopathologic risk profile were linked to the increment of ICU complications, extubating failures and 30 days mortality, such as : (1) decrement systemic lymphocyte count below 724 cells/mm^3; (2) decrement systemic IgG2 below 301 mg/dL; (3) decrement systemic IL-17.[8] Bielosludtseva et al (2013) showed the blood concentration of CD4 T lymphocyte below 500 cells / uL was a predictor for severe pneumonia and related to more complications.[9] Bronchoalveolar lavage fluid (BALF) examination has a role to predict diagnoses entities and local immunopathologic risk profile.[10] Bronchoalveolar lavage fluid has specific diagnostic role especially in interstitial lung disease and sarcoidosis Characteristic of increment CD4/CD8 ratios is a specific findings for sarcoidosis patients .[10,11] However, to the extent of researcher knowledge, there were no observational studies that able to explained the role of local adaptive immunity in pneumonia severe group of patients especially in the pandemic era of COVID-19. We performed a single center, Jakarta, Indonesia, prospective cohort study to assess the role of local adaptive immunity of CD4/CD8 in pneumonia severe with extubation failure and mortality.

METHODS

TRIAL DESIGNS AND OVERSIGHT

We conducted cohort prospective study at Dr. Cipto Mangunkusumo National General Hospital, Jakarta, Indonesia. This is a sub-study from research entitled with “Analysis of Bronchoalveolar Lavage Fluid to assess Local Immunopathologic in Severe Pneumonia (Focus in the Role of sTREM, Alveolar Macrophage, IL-6, IL-17, CD4, T-reg, Surfactant Protein-A, Caspase-3) with ethical clearance number : KET-171/UN.2F1/ETIK/PPM.00.02/2020 and acceptance date on February 17th, 2020.

PARTICIPANTS AND INTERVENTIONS

We enrolled patients who were either actively screened by the researcher team or referred to us who were 18 years of age or

older and has been admitted in emergency room or intensive care unit with severe pneumonia, had antibiotic prescription below 24 hours and in the necessity for mechanical intubation. The patient or the next of kin agreed for the patient to underwent bronchoscopy and bronchial toilet examinations hence the bronchoalveolar lavage fluid can be assessed. Among the reason for exclusions from the trial were the use of antibiotic above 24 hours; refused to underwent mechanical intubation or flexible bronchoscopy procedure; had already been intubated for more than 12 hours; had noninfectious acute respiratory distress syndrome; had HIV/AIDS (screened using anti-HIV ELISA test methods); had active cancer in the past 12 months; and currently using immunosuppressant. All the patients and family provided written informed consent before procedure and well-informed regarding patient condition or outcomes. All of the patients were undergoing mechanical ventilation and bronchoscopy to receive standard care for severe pneumonia. Bronchoalveolar lavage fluids were taken from both side of the lungs started with the lung which has more severe condition based on chest x-ray findings then continued to another side. The current standard of care for pneumonia severe in pandemic era of COVID-19 including the use of anti-viral if indicated, antibiotic, glucocorticoids, and anti-coagulant were allowed. Guidance was provided to the investigators to adjust or interrupt during the procedure of bronchoscopy. There was no mortality observed during procedure of bronchoscopy or endotracheal intubation. Sample were collected and assessed in pathology clinic laboratory of Dr. Cipto Mangunkusumo National General Hospital using flowcytometry methods to acquired the fraction and ratio of CD4/CD8 in bronchoalveolar lavage fluid within 30 hours since sample collection.

OUTCOMES

The primary outcome was extubating failure status within 19 days observations. Extubating failure status were defined as patient's failure in maintained spontaneous breathing trial after extubated hence the patients need to be re-intubated within 72 hours after extubating. Extubating failure status also acquired if patient passed away in intubated condition or within 48 hours after being extubated. Secondary outcomes included mortality status in critical care unit within 28 days observations. All the trial outcomes were assessed by the principal investigator and team who were aware of the research protocol.

Bronchoalveolar lavage fluid were acquired using flexible bronchoscopy with bronchial toilet technique using 100 cc normal saline 0,9% in total after informed consent acquired from the patient or family and patient was sedated, put on analgesic, then intubated prepared for the procedure. The patient had oxygen saturation above 94% using mechanical ventilation machine in 100% fraction of inspired oxygen (FiO_2), pressure-controlled mode prior and during the bronchoscopy procedure which took around 30 – 45 minutes. All of the patients who underwent these studies were well monitored during the procedure and had no mortality or any complications due to minimal invasive flexible bronchoscopy such as pneumothorax or ruptured of trachea/bronchus which could be observed using chest x-ray post-procedure.

Bronchoalveolar lavage fluid were directly stored in the room temperature storage. The samples were processed using flowcytometry technique to detect the

fraction of CD4/CD8 and ratio CD4/CD8 within BALF within 30 hours after bronchoscopy procedure. An amount of 10 ml BALF were put in sterile pot then vortexed to get an homogen sample of BALF. An amount of 50 uL were inserted into BD Trucount™ tube that already been mixed with BD Multitest™ CD45+/CD3+ /CD4+/CD8+ antibody. The mixture within BD TruCount tube were vortexed and incubated in the dark room for 15 minutes. An amount of 450 uL BD FACS™ Lysing Solution were mixed into the tube and had 10 times dilution. The samples were vortexed and assessed using flowcytometry BD FACS CANTO II. The results were analyzed with the bead events and lymphocyte events. The lymphocyte events were divided by bead events times bead counts per sample of BALF volume (50 uL). The results are the count of lymphocyte absolute (CD45+). The results of T cells lymphocyte (CD3+), T cells lymphocyte helper (CD3+4+), and T cells lymphocyte cytotoxic (CD3+8+) were acquired after the percentage of each cells times lymphocyte absolute count (CD45+). The final results were the fraction of T helper lymphocyte cells (CD3+CD4+ per CD45+ [percentage] and its absolute count [cells/uL]) and the fraction of T cytotoxic lymphocyte cells (CD3+CD8+ per CD45+ [percentage] and its absolute count [cells/uL]).

SAMPLE-SIZE CALCULATION

Initially, we planned for the study to include 414 patients, using the cohort prospective analysis study after having pre-liminary calculation of sample size method. However, during the pandemic era, we had shortage in reagent to detect the level of CD4/CD8 T lymphocyte cells hence the study was completed in 40 patients. We calculated that the study would have 55% power to detect the significance mean difference of BALF CD4 T lymphocyte fraction between group based on extubating status; 48.8% power to detect the significance mean difference of BALF CD8 T lymphocyte fraction between group based on extubating status; 16% power to detect the significance mean difference of BALF Ratio CD4/CD8 T lymphocyte between group based on extubating status. We also calculated that the study would have 58% power to detect the significance mean difference of BALF CD4 T lymphocyte fraction between group based on mortality status; 45.9% power to detect the significance mean difference of BALF CD8 T lymphocyte fraction between group based on mortality status; and 1,74 % power to detect the significance mean difference of ratio CD4/CD8 T lymphocyte cells in BALF between group based on mortality status.

STATISTICAL ANALYSIS

Prior to the entry data, the data were evaluated and sorted. The entry data process through coding program of SPSS Statistics 25.0. The investigator has assigned the two-way hypothesis with α 5%, ß 80% with p significance if < 0,005. Univariate analysis used to describe descriptive analysis presented in characteristic table. Basic characteristic compared both group of patients based on extubating status and mortality status. The primary and secondary outcomes were analyzed by numerical mean differences using Mann-Whitney test for abnormal distribution dataset.

RESULTS

CHARACTERISTIC OF THE PATIENTS

There were 137 patients with severe pneumonia. However, only 40 patients with pneumonia severe were recruited which are

fit with the inclusion and exclusion criteria, including 28 patients with confirmed COVID-19. All of the patients were undergoing mechanical ventilation and bronchoalveolar lavage using bronchoscopy to acquire the BALF sample. All of the patients were received standard care of severe pneumonia including intensive care unit admission, antibiotic, anti-viral for confirmed COVID-19, anticoagulant, and mechanical ventilation. The first patient underwent bronchoscopy on November 24th, 2020; the last patient underwent bronchoscopy on January 21st, 2021 and follow up was over on February 18th, 2021. After 19-day follow up of extubation status and 28-day follow up of mortality status, there were 40 patients remained in this study.

The characteristic of the patients is shown in **Table 1, Table 2** based on extubating status, and **Table 3** based mortality status. Most patients had the proportion of extubating failure and mortality outcome (sequentially 32 patients (80%) had extubating failure outcome within 19 days of critical care admission and 30 patients (75%) had mortality outcome within 28 days of critical care admission). The mean age of the patient was 60 years old and 53 % of all included patients were men. All of the 40 patients were severe pneumonia patients whom requiring mechanical ventilation. The median of length of intubation period were 6 days with interquartile range 1 – 65 days. Twenty eight patients within this study were COVID-19 confirmed cases and 10 out of 28 patients had secondary bacterial infection based on BALF culture with the bacteria consist of *Streptococcus alpha-hemoliticus, Enterococcus faecalis, Staphylococcus saphrophyticus, Acinetobacter sp.,* and *Staphylococcus epydermidis.* There were 2 patients who had lung tuberculosis as severe pneumonia etiology within this study.

Table 1 Characteristic of Patients (Demographic, Clinical Condition, Laboratory Result)

Characteristic	All patients n = 40 (100%)
Gender (%)	
Male	21 (52,5)
Female	19 (47,5)
Age, mean (SD), years	59,93 (SB 10,8)
BMI, mean (SD), kg/m^2	26,7 (SB 3,2)
Extubating failure proportions for 19 days observations in critical care ward	32 (80%)
Mortality proportions during for 28 days observation in critical care ward	30 (75%)
Length of stay, days, median (IQR)	7 (1 – 65)
Length of Intubation period, median(IQR)	6 (1 – 65)
History of smoking	8 (20%)
Etiologies[a]	
Pneumonia COVID-19	28 (70%)
COVID-19 + Steril Sputum's culture results	18 (64,3%)
COVID-19 + Streptococcus Alfahemolitikus	2 (7,1%)
COVID-19 + Enterococcus Faecalis	2 (7,1%)
COVID-19 + Staphylococcus saphrophyticus	2 (7,1%)
COVID-19 + Acinetobacter Sp.	2 (7,1%)
COVID-19 + Staphylococcus Epidermidis	1 (3,5%)
Bacterial Pneumonia	18 (45%)
Streptococcus alfahemolitikus	7 (17,5%)
Staphylococcus aureus	3 (7,5%)
Enterococcus faecalis	2 (5%)
Staphylococcus saprophyticus	2 (5%)
Acinetobacter sp.	2 (5%)
Staphylococcus epidermidis	2 (5%)
Lung Tuberculosis	2 (5%)
Commorbidity	
Chronic Obsturctive Pulmonary Disease	2 (5%)
Asthma	2 (5%)
Diabetes Mellitus	22 (55%)
Hipertension	20 (50%)
Neurologic disorder	2 (5%)
Chronic Kidney Disease	18 (45%)
Heart Disease	2 (5%)
Autoimmune disease	1 (2,5%)
Scoring system	
Score APACHE II, median (IQR)	16 (10 – 32)
Skore mSOFA, median (IQR)	9 (6 – 13)
Charlson Commorbidity Index, mean (SD)	4,68 (SB 2,11)
Parameter Laboratorium	
Hemoglobin, g/dL median (IQR)	13,2 (6,0 – 17,3)
Leucocyte, x 10^3 cells /mcL, median (IQR)	13,09 (4,07 – 36,78)
Platelet, x 10^3 cells / mcL, median (IQR)	277,5 (73,0 – 727,0)
ESR, mm/hours median (IQR)	61 (5-130)
CRP, mg/L median (IQR)	125 (1,3 – 650)
Ferritin, ng/mL median (IQR)	1631 (88 – 13.077)
Procalcitonin, ng/mL median (IQR)	0,48 (0,1 – 57,8)
HbA1c, % median (IQR)	6,2 (5,0 – 14,9)
D-dimer, mcg/L median (IQR)	4.025 (440-35.200)
Creatinine, mg/dL median (IQR)	1,3 (0,6 – 20,0)
SGPT, U/L median (IQR)	38 (6 – 216)
Albumin, mg/dL mean (SD)	3,01 (SB 0,53)
PaO2/FiO2 Ratio, median (IQR)	63,6 (17,68 – 338,3)
Blood absolute CD4+ count, cells/mcL median (IQR)	231,5 (32 – 904)
Blood absolute CD8+ count, cells /mcL, median (IQR)	155,5 (22,0-1,354)
Blood Ratio of CD4/CD8 T-cells lymphocyte, median(IQR)	1,59 (0,10 – 4,6)
Fraction of CD4+ BALF of lung with severe injury, % median (IQR)	16 (4 - 174)
Fraction of CD8+ BALF of lung with severe injury, %, median (IQR)	14 (4 – 99)
Ratio of CD4/CD8 BALF of lung with severe injury, median (IQR)	1,05 (0,13 – 3,48)

Table notes: numerical data with normal distribution presented with means and standard of deviation (SD). Meanwhile, numerical data with abnormal distribution presented with median and interquartile range (IQR). Categorical data presented as absolute count and percentage. APACHE = *acute physiology and chronic health evaluation; CD = Cluster Differentiation* ; CRP = *C-Reactive Protein ;* COVID-19 = *Corona Virus Disease 19 ;* HIV = *Human Immunodeficiency Virus;* ESR = Erythrocyte Sediment Rate; mSOFA = *modified sequential organ failure assessment;*

[a] Few patients have more than one etiologies

Table 2. Characteristic of patients based on extubating status

	Extubating success (n=8)	Extubating failure (n=32)
Gender		
Male	4 (50%)	17 (53,1%)
Age, mean (SD), years	61,8 (SD 8,8)	59,44 (SD 11,3)
Indeks Massa Tubuh, rerata (SD), kg/m^2	27,8 (SD 3,6)	26,4 (SD 3,1)
Length of stay, days, median (IQR)	19 (10 – 45)	5 (1 – 65)
Length of intubation, days, median (IQR)	11 (5-19)	6 (1 – 65)
History of smoking		
Yes	0 (0%)	8 (25%)
No	8 (100%)	24 (75%)
Etiologies[a]		
COVID-19[a]	5 (62,5%)	23 (71,9%)
COVID-19 + Sterile BALF Culture[a],	3 (37,5%)	15 (46,8%)
COVID-19 + Streptococcus Alpha-hemolyticus	1 (12,5%)	1 (3,1%)
COVID-19 + Enterococcus Faecalis	0 (0%)	2 (6,2%)
COVID-19 + Staphylococcus saphrophyticus	0 (0%)	2 (6,2%)
COVID-19 + Acinetobacter Sp.	1 (12,5%)	1 (3,1%)
COVID-19 + Staphylococcus Epidermidis	0 (0%)	2 (6,2%)
Bacterial Pneumonia[a]		
Streptococcus alpha-hemolyticus	4 (50%)	3 (9,4%)
Staphylococcus aureus	0 (0%)	3 (9,4%)
Enterococcus faecalis	0 (0%)	2 (6,3%)
Staphylococcus saprophyticus	0 (0%)	2 (6,3%)
Acinetobacter sp.	1 (12,5%)	1 (3,1%)
Staphylococcus epidermidis	0 (0%)	2 (6,3%)
Lung Tuberculosis	1 (12,5%)	1 (3,1%)
Comoridities		
COPD	0 (0%)	2 (6,3%)
Asthma	1 (12,5%)	1 (3,1%)
Diabetes Mellitus	5 (62,5%)	17 (53,1%)
Hipertension	5 (62,5%)	15 (46,9%)
Neurologic Disorder	0 (0%)	2 (6,3%)
Heart Disease	1 (12,5%)	1 (3,1%)
Autoimmune disease	0 (0%)	1 (3,1%)
Chronic Kidney dsease	2 (25,0%)	16 (50%)
Scoring System		
Score APACHE II, median (IQR)	14,0 (10 – 19)	16,0 (10 – 32)
Score mSOFA, median (IQR)	8,0 (8 – 13)	9,0 (6 – 13)
Charlson Commorbidity Index, mean (SD)	4,75 (SD 2,18)	4,66 (SD 2,13)
Laboratory Parameters		
Hemoglobin, g/dL median(IQR)	12,9 (10,2 – 15,9)	12,9 (6,0 – 17,3)
Leucocyte, x 10^3 cells/mcL median (IQR)	15,77 (9,58 – 27,56)	13,02 (4,07 – 36,78)
Platelet, x 10^3 cells/mcL median (IQR)	326,62 (73,00- 727,00)	277,06 (106,00 – 592,00)
ESR, mm/hours, median (IQR)	76 (11 – 130)	56 (5 – 125)
CRP, mg/L, median (IQR)	118,6 (18 – 650)	125 (1,3 – 495,5)
Ferritin, ng/mL, median (IQR)	3.078 (385 – 13.077)	1.613 (88 – 7.095)
Procalcitonin, ng/mL, median (IQR)	0,63 (0,1 – 27,0)	0,485 (0,1 – 57,8)
HbA1c, % median (IQR)	8,45 (5,5 – 13,5)	5,9 (5,0 – 14,9)
D-dimer, mcg/L, median (IQR)	10.260 (3.030 – 35.200)	1.960 (440 – 35.200)
Creatinine, mg/dL, median (IQR)	1,1 (0,7- 2,1)	1,45 (0,6 – 20,0)
SGPT, U/L, median (IQR)	39,0 (9,0 – 196,0)	37,5 (6,0 – 216,0)
Albumin, mg/dL, mean (SD)	2,93 (SD 0,44)	3,24 (SD 0,54)
PaO2/FiO2 Ratio, median (IQR)	94,4 (30,0 – 338,3)	88,12 (17,6 – 242,8)
Blood Absolute CD4 cells count, cells/uL, median(IQR)	332 (52 – 781)	181 (32 – 904)
Blood Absolute CD8 cells count, cells/uL, median(IQR)	137 (56 – 351)	163,5 (22 – 1354)

Table 3 Characteristic of patients based on mortality status

	Survived (n=10)	Death (n=30)
Gender		
Male	5 (50%)	16 (53,1%)
Age, mean (SD), years	60,4 (SB 8,4)	59,77 (SB 11,6)
Indeks Massa Tubuh, rerata (SD), kg/m^2	28,0 (SB 3,8)	26,2 (SB 3,0)
Length of stay, days, median (IQR)	22 (10 – 65)	5 (1 – 20)
Length of intubation, days, median (IQR)	12 (5-65)	5 (1 – 20)
History of smoking		
Yes	0 (0%)	8 (26,7%)
No	10 (100%)	22 (73,3%)
Etiologi[a]		
COVID-19[a]	6 (60,0%)	22 (73,3%)
COVID-19 + Sterile BALF Culture[a],	3 (30,0%)	15 (50,0%)
COVID-19 + Streptococcus Alpha-hemolyticus	1 (10,0%)	1 (3,3%)
COVID-19 + Enterococcus Faecalis	0 (0%)	2 (6,6%)
COVID-19 + Staphylococcus saphrophyticus	0 (0%)	2 (6,6%)
COVID-19 + Acinetobacter Sp.	2 (20%)	0 (0 %)
COVID-19 + Staphylococcus Epidermidis	0 (0%)	2 (6,6%)
Bacterial Pneumonia[a]		
Streptococcus alpha-hemolyticus	4 (40%)	3 (10,0%)
Staphylococcus aureus	0 (0%)	3 (10,0%)
Enterococcus faecalis	0 (0%)	2 (6,7%)
Staphylococcus saprophyticus	0 (0%)	2 (6,7%)
Acinetobacter sp.	2 (20,0%)	0 (0%)
Staphylococcus epidermidis	0 (0%)	2 (6,7%)
Lung Tuberculosis	1 (10,0%)	1 (3,3%)
Comoridities		
COPD	0 (0%)	2 (6,7%)
Asthma	1 (10,0%)	1 (3,3%)
Diabetes Mellitus	7 (70,0%)	15 (50,0%)
Hipertension	6 (60,0%)	14 (46,7%)
Neurologic Disorder	0 (0%)	2 (6,7%)
Heart Disease	1 (10,0%)	1 (3,3%)
Autoimmune disease	0 (0%)	1 (3,3%)
Chronic Kidney dsease	3 (30,0%)	15 (50%)
Scoring System		
Score APACHE II, median (IQR)	14,00 (10 – 24)	16,0 (10 – 32)
Score mSOFA, median (IQR)	8,0 (8 – 13)	9,0 (6 – 13)
Charlson Commorbidity Index, mean (SD)	4,80 (SB 1,98)	4,63 (SB 2,18)
Laboratory Parameters		
Hemoglobin, g/dL median(IQR)	13,1 (7,2 – 15,9)	13,2 (6,0 – 17,3)
Leucocyte, x 10^3 cells/mcL median (IQR)	13,20 (9,58 – 27,56)	13,02 (4,07 – 36,78)
Platelet, x 10^3 cells/mcL median (IQR)	305,50 (73,00 - 727,00)	272,00 (106,00 – 592,00)
ESR, mm/hours, median (IQR)	98 (11 – 130)	51,5 (5 – 125)
CRP, mg/L, median (IQR)	136,5 (18 – 650)	122,7 (1,3 – 495,5)
Ferritin, ng/mL, median (IQR)	2.697 (385 – 13.077)	1.613 (88 – 7.095)
Procalcitonin, ng/mL, median (IQR)	0,63 (0,1 – 27,0)	0,485 (0,1 – 57,8)
HbA1c, % median (IQR)	8,15 (5,5 – 13,5)	5,9 (5,0 – 14,9)
D-dimer, mcg/L, median (IQR)	10.260 (980 – 35.200)	1.960 (440 – 35.200)
Creatinine, mg/dL, median (IQR)	1,1 (0,7- 10,5)	1,45 (0,6 – 20,0)
SGPT, U/L, median (IQR)	37,0 (9,0 – 196,0)	38,5 (6,0 – 216,0)
Albumin, mg/dL, mean (SD)	2,92 (SB 0,41)	3,27 (SB 0,54)
PaO2/FiO2 Ratio, median (IQR)	92,7 (30,0 – 338,3)	61,2 (17,6 – 242,8)
Blood Absolute CD4 cells count, cells/uL, median(IQR)	397 (52 – 781)	153 (32 – 904)
Blood Absolute CD8 cells count, cells/uL, median(IQR)	172 (56 – 351)	149 (22 – 1354)

PRIMARY OUTCOME

The primary outcomes (mean differences of the fractions of T cell lymphocyte CD4/CD8 and ratio CD4/CD8 in BALF based on extubating failure status) was assessed in all patients who were still admitted in critical care unit within 19 days observations. All the patients were severe pneumonia patients whom treated with mechanical ventilation, there were significantly differences of the fraction of CD4 T cell lymphocyte in BALF between the group of patients who successfully extubated compared to the group of patients who unsuccessfully extubated (p=0,006). Meanwhile, there were no significantly differences of the fraction of CD8 T lymphocytes cells in BALF (p=0,063) and ratio of CD4/CD8 T lymphocytes cells in BALF among the group of patients who successfully extubated versus unsuccessfully extubated (p=0,467). The median of fraction and ratio CD4/CD8 T lymphocytes were shown in **Table 4.**

SECONDARY OUTCOME

The secondary outcomes (median differences of the fractions of T cell lymphocyte CD4/CD8 and ratio CD4/CD8 in BALF based on mortality status) was assessed in all patients who were still admitted in critical care unit within 28 days observations. All the patients were severe pneumonia patients whom treated with mechanical ventilation, there were significantly differences of the fraction of CD4 T cell lymphocyte in BALF between the group of patients who survived compared to the group of patients who death (p=0,002). Meanwhile, there were no significantly differences of the fraction of CD8 T lymphocytes cells in BALF (p=0,062) and ratio of CD4/CD8 T lymphocytes cells in BALF among the group of patients who survived versus death group of patients (p=0,158). The median of fraction and ratio CD4/CD8 T lymphocytes were shown in **Table 5.**

Table 4. Median difference of fraction T -cells lymphocyte of BALF from severely injured lung based on extubating status from 19 days observations in critical care ward

	Extubating Success (n = 8)	**Extubating Failure (n = 32)**	***p-value*[a]**
CD4 BALF, %, median (IQR)	43,5 (16 – 102)	12 (4 – 174)	**0,006***
CD8 BALF, %, median (IQR)	29,5 (10 – 99)	13,5 (4 – 64)	0,063
Ratio of CD4 / CD8 BALF, median (IQR)	1,30 (0,48 – 3,40)	1,18 (0,13 – 3,48)	0,467

[a]Statistical test *Mann-Whitney* for numerical data with abnormal data distribution. P significance if < 0.05

Table 5. Median difference of fraction T-cells lymphocyte of BALF from severely injured lung based on mortality status from 28 days observations in critical care ward

	Survived (n = 10)	**Death (n = 30)**	***p-value*[a]**
CD4 BALF, %, median (IQR)	43,5 (14-146)	22,5 (4-174)	**0,002***
CD8 BALF, %, median (IQR)	29,5 (10-99)	13,5 (4-64)	0,062
Ratio CD4 / CD8 BALF, median (IQR)	1,39 (0,48 – 3,40)	1,00 (0,13-3,48)	0,158

[a]Statistical test *Mann-Whitney* for numerical data with abnormal data distribution. P significance if < 0.05

Additional analysis was made to study the median differences of fractions and ratio of CD4/CD8 T lymphocytes cells between the right and left lung. This additional analysis goal was determined to picture the role of local adaptive immunopathology in severe pneumonia patients based on the location of the lung. There were no significantly

differences in the median of the fraction and ratio of CD4/CD8 T lymphocytes cells in BALF either in group of patients with 19-days observation in critical care based on extubating failure status or 28-days observation in critical care based on mortality status. Further, detail results of this analysis were showed **in Table 6 and 7.**

Table 6 Difference between fraction T -cells lymphocyte of BALF based on extubating status and lung site from 19 days observations in critical care ward

	Extubating Success (n = 8)	**Extubating Failure (n = 32)**	***p-value*[a]**
CD4 BALF of left lung, %, mean (SD)	39,75 (SD 13,20)	37,93 (SD 11,94)	0,709
CD4 BALF of right lung, %, mean (SD)	37,50 (SD 12,15)	34,50 (SD 11,86)	0,528
CD8 BALF of left lung, %, mean (SD)	34,62 (SD 14,24)	35,84 (SD 11,62)	0,801
CD8 BALF of right lung, %, mean (SD)	32,75 (SD 12,87)	33,75 (SD 14,47)	0,859
Ratio CD4 / CD8 BALF of left lung, mean (IQR)	1,14 (0,41 – 3,40)	1,00 (0,24 – 6,54)	0,735
Ratio CD4 / CD8 BALF of right lung, mean (IQR)	1,27 (0,40 – 3,00)	1,01 (0,14 – 7,00)	0,678

[a]Statistical test of *Mann – Whitney* for abnormal distribution data and non-paired T-test for equal variance, significance difference if $p < 0.05$

Tabel 7 Difference between fraction T -cells lymphocyte of BALF based on mortality status and lung site from 28 days observations in critical care ward

	Survived (n = 10)	**Death (n = 30)**	***p-value*[a]**
CD4 BALF of left lung, %, mean (SD)	41,90 (SD 12,63)	37,10 (SD 11,83)	0,281
CD4 BALF of right lung, %, mean (SD)	40,00 (SD 11,98)	33,46 (SD 11,50)	0,132
CD8 BALF of left lung, %, mean (SD)	33,50 (SD 12,92)	36,30 (SD 11,82)	0,530
CD8 BALF of right lung, %, mean (SD)	31,50 (SD 12,85)	34,23 (SD 14,52)	0,600
Ratio CD4 / CD8 BALF of left lung, mean (IQR)	1,29 (0,41 – 3,40)	0,95 (0,24 – 6,54)	0,303
Ratio CD4 / CD8 BALF of right lung, mean (IQR)	1,27 (0,40 – 3,30)	1,01 (0,14 – 7,00)	0,463

[a] Statistical test of *Mann – Whitney* for abnormal distribution data and non-paired T-test for equal variance, significance difference if $p < 0.05$

Additional analysis on the fraction and ratio of CD4/CD8 T lymphocytes cells in blood was made to picture the role of systemic adaptive immunopathology in severe pneumonia patients. There were significantly differences of the fraction of the CD4 T lymphocytes cells in blood between group of patients who successfully extubated vs unsuccessfully extubated (p=0,034) for 19 days critical care admission and group of patients who survived vs death (p=0,008) for 28 days critical care admission. The ratio of CD4/CD8 T lymphocytes cells in blood were significantly differences between the group of patients who successfully extubated vs unsuccessfully extubated (p=0,016) for 19 days critical care admission and the group of patients who survived vs death (p=0,011) for 28 days critical care admission. While, the fractions of CD8 T lymphocytes cells in blood was not significantly differences neither in group of patients based on 19 days observation in critical care of extubating failure status (p=0,252) nor 28 days observations in critical care based on mortality status (p=0,344). Further, detail results of this analysis were shown in **Table 8 and Table 9.**

Table 8 Difference of Fraction of Blood T-cells lymphocyte based on extubating status for 19 days observation in critical care admission

	Success Extubating (n = 8)	Failure Extubating (n=30)	p-valuea
Fraction of Blood CD4, %, mean (SD)	35,1 (SD 16,4)	24,5 (SD 10,7)	**0,034***
Absolute cells count of Blood CD4, cells/mcL, median (IQR)	332 (52 – 781)	181 (32 - 904)	0,111
Fraction of Blood CD8, %, mean (SD)	15,3 (SD 6,7)	20,3 (SD 11,5)	0,252
Absolute cells count of Blood CD8, cells/mcL, median (IQR)	137 (56 – 351)	163 (22 – 1354)	0,567
Ratio of blood CD4/CD8, median (IQR)	2,65 (0,43 – 4,60)	1,41 (0,10-3,10)	**0,016***

[a] Statistical test of *Mann – Whitney* for abnormal distribution data and non-paired T-test for equal variance, significance difference if $p < 0.05$

Table 9 Difference of Fraction of Blood T-cells lymphocyte based on mortality status for 28 days observation in critical care admission

	Survived (n = 10)	Death (n=28)	p-value
Fraction of Blood CD4, %, mean (SD)	35,5 (SB 14,52)	23,5 (SB 10,49)	**0,008***
Absolute cells count of Blood CD4, cells/mcL, median (IQR)	397 (52 – 781)	153 (32 – 904)	**0,019***
Fraction of Blood CD8, %, mean (SD)	16,5 (SB 6,51)	20,3 (SB 11,90)	0,344
Absolute cells count of Blood CD8, cells/mcL, median (IQR)	172 (56-351)	149 (22-1354)	0,921
Ratio of blood CD4/CD8, median (IQR)	2,2 (0,43– 4,6)	1,38 (0,10-3,10)	**0,011***

[a] Statistical test of *Mann – Whitney* for abnormal distribution data and non-paired T-test for equal variance, significance difference if $p < 0.05$

DISCUSSION

In this prospective cohort study, it was found that the fraction of CD4 T-cells lymphocyte in BALF was significantly higher either in group of patients with severe pneumonia whom successfully extubated or survived. The fraction of CD8 T lymphocyte in BALF and ratio of CD4/CD8 T lymphocyte in BALF either in group of severe pneumonia patients whom successfully extubated or unsuccessfully extubated were not significantly different as well as the group of severe pneumonia patients based on mortality outcome.

Adaptive local immunopathology has a critical role in pneumonia especially to many pathogens either virus or bacterial. CD8+ T-cell immunity has a limited role in bacterial infection and CD4+ T-cells has a clear role both either in viral or bacterial infection. As patient with HIV infection having a depletion of CD4+ T-cells lymphocyte thus made the patient would suffered from AIDS and has opportunistic infections including *Pneumoncystis jirovecii,* Tuberculosis, Bacterial Infection and Cryptococcal infection.[12] In severe pneumonia with acute respiratory distress syndrome, there is an increasing of inflammatory responses within the lumen of bronchoalveolar which causing rapid onset infiltrative process in both lung parenchyma.[13]

In this study, BALF sample was taken before the patient was on mechanical ventilator before 12 hours and the patient had intravenous antibiotic before 24 hours. HIV/AIDS, immunosuppressant user, 1-year history of active cancer, and patient / family who refuse mechanical ventilation or bronchoscopy were excluded to reduce the bias. T-helper cells and T-cytotoxic cells were human T-cells lymphocyte that express

cluster determinant 4/8 (CD4/CD8) molecules. They were the member of immunoglobulin family which able to primarily mediates adhesion to MHC molecules. HIV/AIDS infection target this CD4 T cells and causing a rapid impairment and deterioration of CD4 T cells. By affecting those mechanism that required for proper CD8+ T-cell maturation, HIV was able to decrease effector and memory CD8+ T-cells. Thus, there's an aberration of CD8+ T-cell function.[14,15] Immunosuppressant were able to inhibit B-cells and T-cells function thus causing the alteration of its differentiation in systemic or peripheral organ.[16] An active cancer can weaken the immune system through interfering the production of white blood cells in bone marrow especially in lymphoma or leukemia cases. High regulatory T-cells were associated with poor prognosis of several cancer such as breast cancer, melanoma, ovarian cancer, and non-small cell lung cancer.[17] However, the effects of antibiotics on the adaptive immunity either in systemic or local were still not well characterized.[18]

Smith et al (2006) showed that lung T-cells lymphocyte phenotype through BALFassessment was useful to evaluate the etiologies of interstitial lung diseases.[19] Ratio of CD4/CD8 was used for the additional diagnosis for sarcoidosis.[20] However, its usage on severe pneumonia were not well established. Bielosludtseva et al (2013) and Fazralimanda et al (2020) had shown that lower concentration of CD4 T-cells lymphocyte and CD8 T-cells lymphocyte in blood was a weak predictor for mortality outcome and severe complication.[9,21] Fazralimanda et al (2020) showed that the absolute count of blood CD4 T-cells lymphocyte below 406 cells/uL was a weak mortality predictor (AUC 0,651; p=0,01) and CD8 T-cells lymphocyte below 263 cells/uL was a weak mortality predictor (AUC 0,639; p=0,018).[21] Up to date, there were still no established study that described the local immunopathology activities in severe pneumonia cases especially during pandemic era of COVID-19. This study showed that the fraction of CD4 T-cells lymphocyte within BALF were significantly higher in group of severe pneumonia patients whom survived and successfully extubated. This study also shown that blood CD4 T-cells fraction and ratio of CD4/CD8 T-cells were significantly higher in group of severe pneumonia patients who survived and successfully extubated.

Analysis on local immunopathology of fraction and ratio CD4/CD8 T-cells lymphocyte in BALF based on lung location in group of severe pneumonia group of patients were similar either in survived or death group of patients. Furthermore, in the group of severe pneumonia patients whom successfully extubated or not, the local immunopathology of fraction and ratio CD4/CD8 T-cells in BALF were similar. Those findings were the novelty from this study. To the extent of the researcher knowledge, there were no previous study had observed this local adaptive immunopathology in BALF for severe pneumonia patients especially during COVID-19 pandemic era. Mucosal immunity has specific role in the defends mechanism in severe pneumonia. Most viruses were using host mucosal surface as their *porte d'entrée* of infection.[22] The respiratory tract mucosal surfaces was the second largest in the body and has a unique characteristic.[22] The mucosal acquired immune system consist of inductive and effector site. The inductive site was associated with mucosal associated lymphoid tissue (MALT) which triggered by mucosal introduced antigens and taken up from luminal. These antigens were

processed and presented by antigen presenting cells to immunocompetent cells such as naïve T-lymphocytes. Finally, a local immunopathology of cellular network consist of Th1, Th2, Th17, Treg, Cytotoxic T cells, B cells, and dendritic cells with epithelial cells would provide the appropriate defensives responses based on locally affected lung.[22] The primary outcome of this study was to compared the local concentration and ratio of CD4/CD8 T-cells lymphocyte from bronchoalveolar lavage fluid from the lung that was severely affected between group of patient based on their extubating status and mortality status. The finding from this study suggest that, it is best to take the BALF sample from the lung site which was severely affected by pneumonia based on radiographic finding (chest xray prior bronchoscopy procedure).

There are several limitations from our trial. First, this study did not differentiate its extubating status based on specific outcome such as simple extubating, difficulty extubating, and prolonged extubating which made the proportion of the patients with extubating failure became higher. Second, this study did not evaluate others viral etiology beside COVID-19 that could cause pneumonia. Third, the study population were limited to the severe-critical pneumonia patient who need to be intubated and did not represent the group of severe pneumonia patients who did not intubated. However, this prospective cohort study can be used as the cornerstone for further study to learn the local immunopathology responses especially in severe pneumonia cases during COVID-19 pandemic era. Further studies are needed to understand the role of Natural Killer cells, Immunoglobulin and antigen presenting cells such as alveolar macrophage and dendritic cells in the severe pneumonia population to predict the outcome of extubating failure and mortality.

CONCLUSION

Fraction of CD4 BALF in severely injured pneumonia lungs group of patients who had successful intubation processes were statistically different compared to the group of patients with unsuccessful extubating. Fraction of CD4 BALF were also found statistically different in the group of patients who were survived compared to the group of patients who were passed away.

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